The Microbial Decomposition of Six Species of New Zealand Bryophytes

Thesis submitted in accordance with the requirements of the University of Canterbury for the degree of Master of Science in Microbiology

By

Stephanie E. Walters

2001

Contents

List of Figures

List of Tables

Abstract

Nutrient cycling and energy flow within various ecosystems has been the subject of many studies in the past. The influence bryophyte litter has on nutrient cycling has not been investigated extensively and research on nitrogen flow, in particular, is limited. In the following study the litter quality and decomposition characteristics of material from six New Zealand moss species was investigated using both laboratory and field studies. *Ptychomnion aciculare, Hypnodendron comatum* and *Dicranoloma dicarpum* were collected from Maruia Springs in the Lewis Pass Reserve and *Hypnum cupressiforme, Breutelia pendula* and *Racomitrium pruinosum* came from Cass near Arthur's Pass.

Differences between the six species and between the green (younger) and brown (older) fractions of the litter were investigated with respect to litter quality, decay rates and nitrogen mobilisation. Due to the lack of an obvious senescence cycle naturally dead moss litter was difficult to determine therefore comparisons between the older and younger material were made.

Moss litter is thought to be resistant to microbial degradation due to the chemical characteristics of the material. Chemical analysis of the species showed a range of high C:N and C:P ratios (53-188 and 206-815 respectively), and that approximately 90% of the acid soluble nitrogen was derived from amino acids and proteinaceous compounds. The high percentage of amino acid and proteinaceous nitrogen indicates that the litter may be of high quality if the nitrogen is not complexed with other compounds causing it to become recalcitrant.

Decay rates (k) were determined from weight loss experiments giving a range of 0.23-1.3. There was an observed increase in weight loss over time for all the litter types. Nitrogen mineralisation studies indicated that nitrogen was released (generally in the form of ammonium ions) by microbial activity over 180 days incubation. Nitrate was also observed which in some cases exceeded ammonium levels. Leaching of the litter yielded water-soluble compounds including high nitrogen levels. Water-soluble extracts were shown to support microbial growth.

Chapter One: Introduction

If changes are to be predicted or induced in ecosystems, the contribution of all levels of plant life should be investigated. As Watt (1947) indicated: "do we in fact know any of it, unless we know all of it?"

1.1 General ecosystem

Ecosystems contain a number of components and processes, which are important for the communities' efficient functioning. An autotrophic group (via the processes of photosynthesis, water transportation and nutrient uptake) creates biomass. The heterotrophic component, which includes herbivores, carnivores, omnivores and parasites, consumes the biomass for energy and structural components for their own biomass. The decomposer organisms drive the process of decomposition, which coupled with respiration and death releases energy, which may be recycled. The decomposers are capable of remineralising nutrients back to inorganic forms that can be used again in the ecosystem. An opposing function of the decomposers is to take up nutrients, which will decrease their availability to the autotroph component. Other crucial components of the ecosystem are the pools of nutrients (in the detritus component, soil solution), the movement of nutrients (via water, wind, and organism transport) and the inputs to and losses from the system. The term nutrients refer to the elements, which are essential for structure and function of organisms such as N, P. K. Ca. Some of these nutrients, particularly nitrogen, are often limiting in the terrestrial ecosystem. There are four important aspects of nutrient cycling (DeAngelis, 1992)

1) Nutrient limitation of primary and secondary production
2) The recycling of nutrients and the mechanisms involved
3) Chemical complexity
4) Stoichiometry

The areas of importance for the following research are the processes involving decomposition and the impact that the rate of breakdown and release from bryophyte

(refer to section 1.4), specifically moss, material could have on the environments in which they are found.

1.2 Nutrient cycling

Nutrient cycling refers to the movement of nutrients through an ecosystem. This movement is important for the continual replenishment of important nutrients such as nitrogen and phosphorus. Nutrients become locked away in various forms in an ecosystem and therefore there is a need for processes that will release these compounds and allow them to be utilised by other organisms. Nutrient cycling is consequently important to the overall functioning of the ecosystem. Microorganisms (bacteria, fungi) play a large part in these processes. The ability of microorganisms to breakdown various substrates are fundamental to the continuation of the cycle. Knowledge of the processes and organisms involved in nutrient movement through an ecosystem is valuable to the general understanding of the ecosystem and can be used to indicate what effect disturbances in the environment may have on the overall functioning of the system.

Carbon, nitrogen and phosphorus cycles are of particular significance to any environment due to the need for these elements in essential compounds such as proteins, amino acids and nucleotides. Carbon in various forms (such as CO_2, carbonate, and any organic compound) is abundant but nitrogen, in particular, is a necessary element that is not present in large amounts in forms available for uptake. The oxidation reactions that convert organic nitrogenous compounds back to usable inorganic forms are referred to as the process of mineralisation

1.2.1 Mineralisation

Nitrogen mineralisation occurs during the nitrogen cycle and is the process whereby enzymes derived from microorganisms breakdown large organic nitrogenous compounds (such as proteins) into smaller inorganic nitrogen constituents (such as ammonia and nitrate). These smaller inorganic compounds can then be taken up by

plants and incorporated into new plant biomass (Pleczar Jr *et al*, 1993). Mineralisation is an essential function of decomposition for it replenishes the soil solution equilibrium in relation to plant growth demands (Swift *et al*, 1979). The process of mineralisation is accompanied by immobilisation, which occurs when an element is either incorporated into or maintained in an organic form. The key to the availability of inorganic forms of nutrient elements it therefore the net mineralisation, which is the extent to which mineralisation exceeds immobilisation (Swift *et al*, 1979). The extent of net mineralisation of any element relates directly to the availability of that element to the decomposer organisms. Carbon is usually in plentiful supply in the early stages of decomposition but other elements such as nitrogen or phosphorus may only be present in low amounts. These low level nutrients may limit the growth of the decomposer organisms and therefore cause net immobilisation. The opposite situation applies when a nutrient is non-limiting, where net mineralisation will prevail (Swift *et al*, 1979; Richards, 1987).

1.2.2 Nutrient availability

An availability index for elements can be calculated by giving the energy: nutrient ratio, which indicates the limits that energy sources and nutrient concentration have on growth. Commonly the ratio is given as a carbon: nutrient ratio as carbon is broadly proportional to energy accessibility for heterotrophs. The ability of an organism to decompose a substrate rapidly can be indicated by comparing the carbon:nutrient ratios of the organisms with that of their food resources. For a general example wood has a carbon: nitrogen (C:N) ratio of 157 and a carbon: phosphorus (C:P) ratio of 1424 whereas a fungal decomposer has a C:N of 26 and C:P of 94. The decomposer has narrower ratios than the substrate, which indicates a high demand for nutrients therefore the nitrogen and phosphorus availability may limit the rate of decay by the fungal decomposer. The carbon:nutrient ratio will alter as decomposition proceeds, which is due to the continual loss of carbon and the immobilisation of the limiting nutrient. If this situation continues the nutrient will cease to be limiting and net mineralisation can occur (Swift *et al*, 1979). The most commonly used ratio is the C:N ratio and generally a low C:N ratio is associated with a high resource quality (see

section 1.3.2) and rapid rates of decomposition, where high C:N ratios indicate low resource quality and slow decomposition (Killham, 1994).

1.3 Decomposition processes

Decomposition proceeds by means of three processes that result in changes in the chemical structure and reduction in size of the litter. The three processes are 1) leaching, 2) catabolism and 3) comminution.

1) Leaching is an abiotic process where soluble matter is removed from the substrate by the action of water. The result of leaching is a loss in weight and a change in the chemical composition of the leached substrate (Swift *et al*, 1979). Leaching is usually an initial process during decomposition and the nutrients in the leachates are lost rapidly to the soil environment.

2) Catabolism is the energy-yielding metabolic breakdown of complex compounds to smaller or simpler molecules. Aerobic respiration of glucose to carbon dioxide and water is the most familiar example.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

The fate of the products of catabolism varies. Some products will enter the metabolic pool of the decomposer organisms and be resynthesised into components of the decomposer tissues, others may be incorporated in non-cellular organic matter such as humus. Products may also be in forms that will volatilise or be leached out (Swift *et al*, 1979). The rate of catabolism can vary and is dependent on the chemical structure of the substrate.

3) Comminution refers to the reduction in particle size of the substrate. This is a physical process, which generally involves the feeding activity (both ingestion and digestion) of decomposer animals. The process may also be brought about by abiotic factors such as freezing and thawing or wetting and drying cycles (Swift *et al*, 1979).

Three major factors control decomposition: the nature of the decomposer community, the resource quality and the physico-chemical environment.

Figure 1 The central concepts of decomposition of organic matter

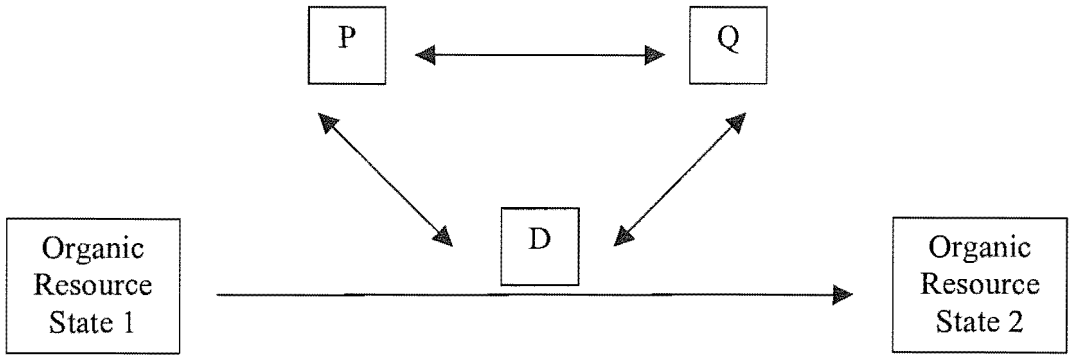


Figure 1 modified from (Heal *et al*, 1997) graphically illustrates the influence the decomposer community (D), resource quality (Q), and physico-chemical environment (P) have on the rate of change of the organic resource over time. The rate of change is regulated by a combination of the three interacting factors. The module represented in Figure 1 is then repeated in a cascade as the primary resource is decomposed and redistributed through the processes of leaching, catabolism and comminution.

1.3.1 Decomposer organisms

The organisms involved in decomposition are a diverse group including many invertebrates, bacteria, and fungi. Some examples of the organisms involved are shown in Table 1 (Swift *et al*, 1979). The litter quality and the physico-chemical environment influence the activity and distribution of these organisms. Changes in microbial biomass during decomposition are usually concomitant with changes in the plant litter quality (Wardle and Laville, 1997). As the chemical environment of the litter changes with decomposition the biomass also changes due to either increased or decreased nutrient availability. The species composition also alters due to the chemical changes occurring in litter. Certain microbial species will be capable of

breaking down and utilising certain substrates, therefore a succession will occur as different compounds are made available for breakdown (Richards, 1987).

Table 1 Decomposer groups and typical organisms associated with decomposition

Group	Organism (common name)
Invertebrate	Protozoa Nematodes Leeches Earthworms Slugs/ snails Termites Beetles
Prokaryote	Cyanobacteria 'True' bacteria Actinomycetes
Fungi	Yeasts Mushrooms Rusts Bracket fungi Zoosporic fungi

1.3.2 Litter and litter quality

Plant litter is described as dead plant matter and consists of material such as leaves, reproductive organs (e.g. cones, flowers and fruit), stems, branches, bark, and roots (Swift *et al*, 1979). Energy flow through a system is closely related to the processes of accumulation and decomposition of organic matter. Litter fall is, therefore, an important component of energy transfer within an ecosystem (Richards, 1987).

The extent of litter accumulation in a system influences the rate of nutrient turnover. If there is a large litter accumulation but it is rapidly decomposed then the nutrient

flow will be unlikely to inhibit plant growth. If litter accumulation exceeds the rate of litter fall the nutrients in the senesced material will be retained in the litter layer.

Litter of different plants does not decompose at similar rates (Williams and Gray, 1974). This is due to the differences in structure and chemical composition (litter quality) of the various regions of the plants. For example it is noted that coniferous trees generally decompose at a slower rate than deciduous trees. There are always exceptions however and it has been shown that non-coniferous plants will also produce litter, which decomposes slowly. For example Latter and Cragg (1967) found leaves of *Juncus squarrosus* (L.) spent approximately 3 years in the litter layers above acid peat, with a dry weight loss of 20-25% in the first year. In comparison *Festuca sylvatica*, a herbaceous species, lost 65-70% of its weight in one year.

The quality of the litter is an influencing factor on the rate at which litter decays Litter quality refers to the chemical composition of the litter such as the C:N and C:P ratios and the lignin content. These factors may affect the rate of breakdown of the litter material. For example Berg and colleagues (1989) in Sweden have demonstrated that for roots and pine needles nutrients such as N, P and S control the rate of decay during the first phase (≤30% mass loss), whereas the lignin content becomes increasingly more important following the first phase (Taylor *et al*, 1989).

1.3.3 Physico-chemical environment

The physico-chemical environment refers to factors such as moisture, pH, temperature and aeration, which can influence the decomposer organisms. These factors can be influential at a micro and macro-environment level (Swift *et al*, 1979). Richards (1987) indicated that decomposition proceeds more rapidly in tropical than temperate climates, which acknowledges the influence the physico-chemical environment has on decomposition.

Moisture within the microhabitat is important for tissue growth and as a medium for aquatic organisms such as protozoa and nematodes. Moisture also indirectly affects pH and aeration. The amount of moisture in the soil and litter environments is

dependent on the quantity of water supplied via precipitation. All the water coming into the environment is not retained in the litter and soil, however, as it can be intercepted by vegetation and lost by evapotranspiration, drainage and lateral run-off (Swift *et al*, 1979). The influence of moisture on litter decay has been demonstrated by a number of workers for example waterlogging of litter can decrease gaseous diffusion and result in anaerobic conditions, which may inhibit decomposition (Williams and Gray, 1974).

Bacteria and fungi are able to modify the pH of their immediate environment. Generally, however, bacteria tolerate a narrower alkaline range of pH in comparison to fungi, which have a broader range but are more active in acidic conditions. The pH may influence the activity of microbial extracellular enzymes and may also affect the availability of essential elements to the decomposer organisms (Swift *et al*, 1979).

The terrain, extent of vegetation and seasonal variation are factors, which affect temperature in an environment (Swift *et al*, 1979). Gradients occur in the upper soil layers with the temperature at the surface often related to the air temperature (Williams and Gray, 1974). The rates of chemical reactions are influenced by temperature with a general relationship of increasing temperature causing a higher reaction rate. Organisms also react to temperature variation with changes in activity. Microorganisms can be grouped by their ability to function in certain temperature ranges. For example thermopiles are adapted to high temperatures and psychrophiles are adapted to low temperatures. The ability of microorganisms to cope with temperature changes by changing the population may be of importance to the overall rate of decomposition. For example in a compost system the temperature may reach levels as high as 70-80°C, which corresponds with a thermophilic microbial population, but at the lower temperatures mesophilic microorganisms dominate. The population shifts in response to the change in temperature, which allows the decomposition process to continue. There is evidence that similar population fluctuations occur between mesophiles and psychrophiles in cold environments (Swift *et al*, 1979).

The processes of decomposition have been extensively studied in laboratory and field experiments using a variety of substrates and techniques to monitor the degradation. These techniques include radioisotope tracking of labelled carbon or nitrogen, nitrogen and carbon mineralisation (releasing ammonium and CO_2) and weight loss

1.4 Bryophytes

Bryophyta is a division of simple plants possessing no vascular tissue (xylem and phloem) and rhizoids instead of a root system (Doyle, 1970). It includes the classes Musci (mosses) and Hepaticae (liverworts) (Beever *et al*, 1992). Bryophytes are found in abundance in moist environments but are they not restricted to this environment, as they also grow in arctic, antarctic and arid regions (Doyle, 1970). Bryophytes have been found to grow on soil, fallen logs, unweathered rocks, roofs and sides of buildings, as epiphytes and occasionally as aquatic plants in fresh water (Doyle, 1970).

1.5 Importance of bryophytes in ecosystem functioning

It is possible that bryophytes (both mosses and liverworts) are over looked in relation to their importance in the functioning of ecosystems. For example in several environments such as deserts, bogs and tundra they often dominate. In more complex ecosystems their biomass may be small in comparison with higher plants but they are capable of having a distinct role despite their low biomass. Weetman (1968) illustrated this point by suggesting that a large proportion of the annual nutritional requirements of spruce trees in a Canadian forest could be met by the decomposition of mosses. However he provided no experimental evidence to support his idea.

Likewise the influence bryophytes have on nutrient availability has not been investigated fully but it is considered to be both significant and distinctive due to the limited grazing, moderate levels of production and often low decomposition rates (Longton, 1992). Bryophytes are likely to aid in nutrient retention within the ecosystem by incorporating nutrients dissolved in precipitation directly into shoot

tissue and by absorbing precipitation into capillary spaces within the moss colonies. This may also reduce leaching of minerals dissolved in soil moisture (Longton, 1984). The retention of nutrients by absorption may be of particular importance during the winter period where higher plants would not be as active in nutrient uptake. The incorporated nutrients could be detained (rather than lost through lack of activity) in the environment until processes such as leaching and decomposition release the compounds back into the nutrient pool (Van Tooren *et al*, 1987). It is also possible that bryophytes increase rates of weathering and therefore nutrient release. In contrast to bryophytes aiding in retention and release of nutrients into the ecosystem, the storage of compounds in living and dead moss material may decrease the availability of nutrients to other organisms (Longton, 1984).

To illustrate the importance bryophytes potentially have in nutrient accumulation, Chapin III *et al* (1987), calculated that feather mosses (mainly *Hylocomium* and *Pleurozium*), which constituted 6% of the total plant biomass of a black spruce forest, contained 17% of the total plant phosphorus pool. Due to the annual production of the mosses equalling 49% of the total plant production (in the ecosystem) the phosphorus contained in the annual growth represented 75% of the total annual accumulation by plants. These calculations suggest that bryophytes may constitute an important reservoir of phosphorus and may have significant impact on nutrient cycling in this habitat, and possibly other habitats such as grasslands (Brown and Bates, 1990). Calculations such as this and that of Weetman (1968) are useful but unfortunately experimental evidence to support these claims is lacking.

1.6 Ecology, chemistry and decomposition of mosses

1.6.1 Ecology of mosses

Mosses are commonly found in woods, fields and shaded stream banks. They are generally associated with moist climates but they are not restricted solely to wet environments (Doyle, 1970). There are a number of truly aquatic species that live submerged in streams and lakes, however there are many drought-resistant species,

which are able to live on arid habitats such as dry exposed rocks (Beever *et al*, 1992). In many of these environments mosses form extensive mats or carpets. All moss species have a generally similar vegetative morphology consisting of a solid axis of cells bearing spirally arranged leaves and usually the capsules (containing the reproductive spores) are borne on stalks (seta) (Round, 1969). The direction the plant grows may vary however as some species grow upright while others have a creeping growth form (Round, 1969). Once the basic vegetative morphology, described by Doyle (1970), has been observed it can be seen that there is actually considerable morphological and physiological variation present with in the class of moss (Doyle, 1970). For example a feature specific to drought-resistant mosses is that they are able to desiccate. Their cells are not damaged when dried out and when water is available they are able to absorb it quickly and start photosynthesising almost immediately (Beever *et al*, 1992). Certain morphology is often associated with different habitats, for example 'umbrella mosses' are only found in moist, shady sites and Sphagnum species will be found where there is abundant water such as in swamps and ditches (Beever *et al*, 1992). There are exceptions to this association such as *Hypnum cupressiforme* and *Thuidium furfurosum*, which grow in open, shaded, dry and damp habitats (Beever *et al*, 1992).

The six species of moss that have been selected for study in the following experiments were representative of the variability amongst the moss group. *Ptychomnion aciculare, Hypnodendron comatum,* and *Dicranoloma dicarpum* are present in the soil and on rotting logs (Beever *et al*, 1992) in Red Beech (*Nothofagus fusca*) forests. The forest is a moist environment that has a higher canopy layer than tussock grasslands, which supplies intermittent light to the ground layer species. *Hypnum cupressiforme, Breutelia pendula* and *Racomitrium pruinosum* exist on rocks and soil in high altitude tussock grasslands (Beever *et al*, 1992) where tree cover is reduced and where the mosses are more exposed to climatic changes such as wet and dry cycles and wind.
In both environments the selected mosses were visually dominant components of the ground cover.

Bryophytes act as habitats for microorganisms, and insects (Gerson, 1982). In some cases moss species participate in mutualistic relationships with nitrogen fixing bacteria (Proctor, 1982) and mychorrizal fungi. It has also been suggested (Keizer *et al*, 1985; Van Tooren *et al*, 1987) that mosses can create favourable and/or unfavourable germination environments for various plant species.

1.6.2 Chemistry of mosses

Bryophytes have been frequently indicated (Longton, 1984) to be unsuitable substrates for many organisms due to aspects of their chemistry. It has been suggested that they possess wider C:N ratios, greater amounts of holocellulose and crude fibre and lower overall energy levels than vascular plants which therefore makes them less appealing as a food source for herbivores (Russell, 1990). "Lignin-like" compounds and polyphenols are also thought to be present in some species of moss (Erickson and Miksche, 1974).

Bryophytes contain chlorophyll and have similar biochemical requirements, mechanisms, and end products as other lower green plants (Frankland, 1974). Despite the chemical similarities between lower green plants, their pattern of decomposition differs depending on their form, composition and habitat. A simple relationship between the morphological complexity of the plant and the rate of decomposition does not seem apparent. This can be illustrated by comparing the slower decomposition of larger land plants due to the increased level of recalcitrant supporting tissues to the resistance of Sphagnum to breakdown which has been described as less complex than higher land plants (Frankland, 1974).

1.6.3 Decomposition of mosses

Plant litter is often defined as "the dead material lying on the soil surface" (Frankland, 1974; Satchell, 1974). This statement does not apply as rigidly to the decomposing material of bryophytes and other cryptogams. This is due to a lack of a distinct decay pattern in bryophytes and other lower plants (Frankland, 1974). Larger plants have a relatively well-defined process of death and decay with bryophytes however it is

usually difficult to find a distinct division between living, senescent and dead portions (Frankland, 1974). For example when the leaves of higher plants senesce and subsequently die there is usually a redistribution of nutrients from the leaf to the main body of the tree to avoid excessive nutrient loss. The leaf then enters the detritus pathway and is rapidly attacked by decomposer organisms and broken down. This cycle is not as obvious when observing the moss plant as the tissue, which imperceptibly becomes brown, may be alive for many years and possibly still contain biologically active compounds (Longton, 1992). It has also been found that dead leaves will also remain attached to a living stem for many years (Frankland, 1974).

In many environments the rate of decomposition is not proportional to the rate of biomass production for bryophytes. Therefore there is often an accumulation of bryophyte biomass, as the decomposition rates are considerably slower than production (Clymo, 1965; Longton, 1984).

This is partly due to limited grazing by animals and insects (Frankland, 1974; Longton, 1984). The lack of herbivory reduces the range of organisms involved in breaking down the litter. Higher plants are generally attacked both by animal and insect groups as well as by the microbial population. In the case of bryophytes the microbial population mainly controls the decay processes although there are exceptions in some environments. For example in the Arctic tundra caribou and reindeer forage on moss species (Prins, 1982) and in temperate and tropical environments evidence of bryophyte consumption involves invertebrates rather than vertebrate grazers (Longton, 1984). Gerson (1982) and Longton (1984) indicated that there are many invertebrate species that feed on bryophytes but that they are often not host specific and may choose to graze on other more easily digestible plant matter if it is available. It is also suggested that although there are some insect and animal species that are capable of digesting bryophyte material there are many that are unable to feed on bryophytes due to production of inhibitory secondary metabolites by the plants (Gerson, 1982). In a study using slugs as generalist herbivores, Davidson *et al* (1990) found that immature capsules (sporocarps) were consumed at a high frequency, whilst shoots were not freely consumed. It has been reported that bryophytes decompose

more slowly than Phanerograms in Sphagnum bogs, temperate forests, Boreal forests and in Arctic tundra (Van Tooren, 1988).

1.7 Past research on moss decomposition

Studies have been undertaken which have concentrated on phosphorus and potassium loss from litterbags at specified times. Nitrogen was looked at in conjunction with the above elements in some of the research but it was not extensively studied. These papers such as (Clymo, 1965; Berg, 1984; Davis, 1986; Smith and Walton, 1986; Van Tooren *et al*, 1987; Van Tooren, 1988; Rochefort *et al*, 1990; Johnson and Damman, 1991) were concerned with weight loss and leaching processes using litterbag experiment data. Van Tooren (1988) found that weight loss showed a sharp decrease from 100% to approximately 80% and 70% at two chalk grassland sites within the first four months of a litterbag experiment. In the following months there was little change in the weight loss. Clymo (1965) also found that when the litterbags were near the surface the loss in weight in the first 4 months was approximately 10%. Berg (1984) indicated that litter of *Dicranum polysetum* had a mass loss of 15% in the first year and after 2 years had only lost 25% of the original mass. After four years in the field approximately 50% of the moss was decomposed as measured by weight loss.

Van Tooren (1988) also measured nitrogen concentration, as a percentage of dry weight, during the experiment. The results indicated a sharp decrease in the first months at both sites, which was probably due to leaching, but then later showed an increase in nitrogen concentration (at differing rates) due to nitrogen accumulation. This immobilization is probably the result of microbial activity and takes place until a certain C/N ratio is reached, after which a final release phase takes place (Van Tooren, 1988).

In reference to the nitrogen content of moss plants Hogg *et al* (1994) found concentrations for *Sphagnum magellanicum* to range from 0.46-1.14% depending on the bog microsite from which the sample was taken. Phosphorus content was also analysed giving results of 0.22-0.44% over the bog microsites. Coulson and

Butterfield (1978) stated that *Sphagnum recurvum* had a nitrogen concentration of 0.78% and a phosphorus concentration of 0.08%. Berg (1984) also found similar nitrogen percentages for *Dicranum polysetum* (0.89%) and *Sphagnum* sp. (0.59%). These previous studies have indicated that bryophytes exhibit a reduced decomposition rate, which may be due to recalcitrant compounds such as polyphenols and other antimicrobial compounds. It has also been suggested that despite the biomass of more complex vascular plants exceeding that of bryophytes in ecosystems such as forests, in other ecosystems such as bogs, mires, and grasslands the bryophyte biomass is significant from a nutritional viewpoint and may have more of an influence on various aspects of the ecosystems functioning than has been considered in the past. Past work has been concentrated mainly in the Northern Hemisphere habitats (with the exception of Antarctica) especially in tundra environments and *Sphagnum* bogs.

Previous research in the area of bryophyte breakdown has commonly used weight loss and total element loss as indicators of the rate and extent of decomposition. Monitoring nitrogen mineralisation has not been used to date in studying bryophyte degradation.

1.8 Objectives

Experimental work is needed to indicate the role and influence moss litter and its decomposition has on the environment.

The main objectives of this study were to:

- Determine the total nitrogen and phosphorus content of moss litter of each species,
- Investigate the mineralisation of moss litter,
- Compare water-soluble content of each species and establish the nitrogen loss via leaching,
- Determine the nitrogen distribution of the litter species, as an indicator of litter quality,
- Investigate the rate of weight loss of moss litter,
- Determine whether leachates from each species could support microbial growth,
- Study decomposition in the field.

Chapter Two: Methods and Materials

2.1 Collection Site Descriptions

The Cass region (Figure 2, near Arthur's Pass) is a high altitude (800-1000m above sea level) rocky site with grasslands (Burrows, 1977) and few large trees. The mosses from this site colonise the soil and rock and form mats, which can extend up to a metre across. The environment is more exposed than a forest system, as there is a lack of extensive canopy cover. Other plant species, which inhabit the Cass site, include matagouri (*Discaria toumatou*), kanuka (*Kunzea ericoides*), manuka (*Leptospermum scoparium*), *Coprosma propinqua*, *Hebe* sp. and various grasses (Shanks *et al*, 1990). Light levels and wind exposure is higher, than the Maruia forest site, due to the lack of canopy cover and snow cover in winter is more prominent.

The Maruia Springs site (Figure 2) is situated in the Lewis Pass Reserve (700-800m above sea level) and is dominated by Red beech (*Nothofagus fusca*) forest. It is generally a wetter climate (average rainfall was approximately 473mm for 2000, NIWA, 2000) than Cass (average rainfall was approximately 177mm for 2000, NIWA, 2000), and the moss species grow on rotting logs or on the ground under the cover of the beech trees. The dominant beech species is Red beech (*Nothofagus fusca*) but Mountain beech (*Nothofagus menziesii*) is also present. The forest is an old-growth stand. The mosses present at this site generally grow in moist habitats and differ in form from the Cass species, which are more adapted to arid, exposed habitats.

Three of the most common species observed (visually) at each site were collected. The plants were subsequently air-dried (for a week) and any extraneous debris was cleaned from the plants.

2.2 Species Descriptions

Allan Fife at Landcare Research, Lincoln, identified the mosses to species level.

Maruia

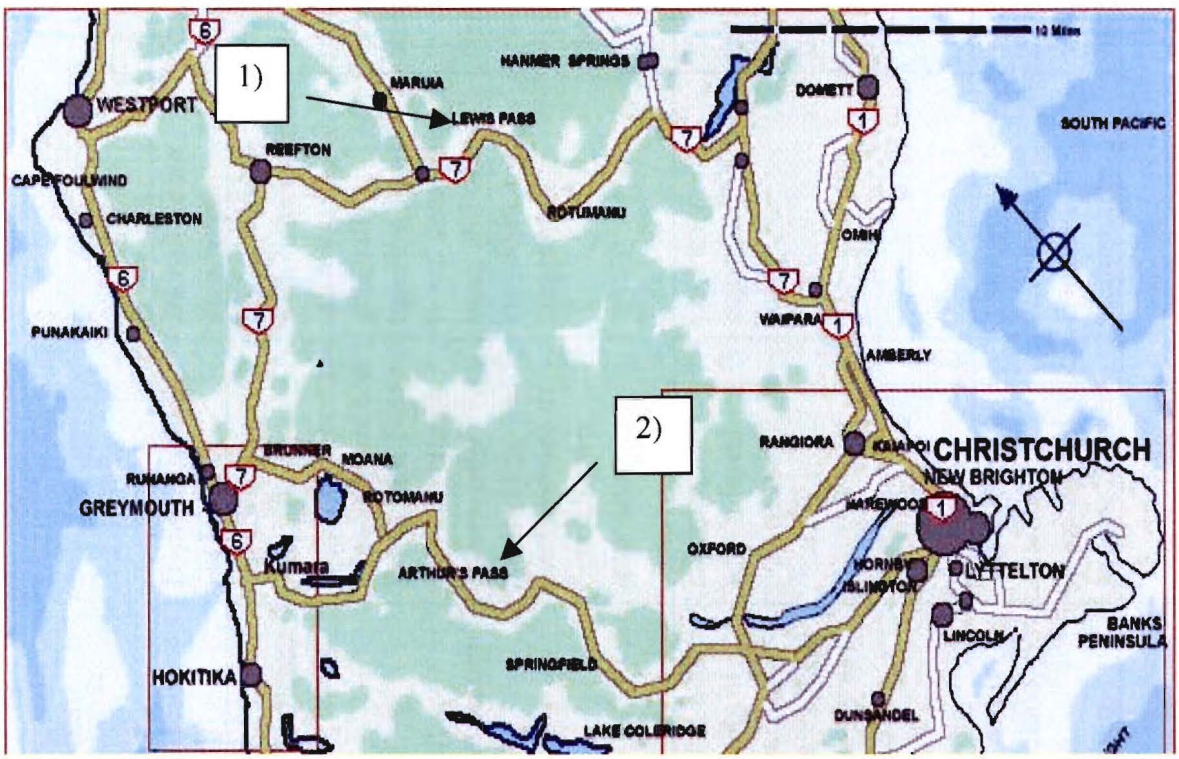
Ptychomnion aciculare, Hypnodendron comatum, Dicranoloma dicarpum

Cass

Hypnum cupressiforme, Breutelia pendula, Racomitrium pruinosum

Refer to Table 2 for morphology, habitat and distribution of each species (Beever, 1992).

Figure 2 Map showing the location of the two collection sites



The two arrows indicate the two collection sites, 1) The Red beech forest in the Lewis Pass Reserve and 2) Cass field station near Arthur's Pass.

Table 1 Species description

	Ptychomnion aciculare	*Hypnodendron comatum*	*Dicranoloma dicarpum*	*Hypnum cupressiforme*	*Breutelia pendula*	*Racomitrium pruinosum*
Appearance	Red stems 5-10cm tall, glossy spreading leaves. Leaves irregularly crumpled and papery in texture when dry	"Umbrella moss", with "fluffy" appearance due to densely branched frond with finely tapering branch leaves. Stipe leaves large and partly buried in dense brown tomentum,.	Large mosses with leaves which are long, gradually tapering from broad sheathing base, and often curved in a crescent	Robust, it may have a bronze tint, or may be green. Leaves strongly curved to one side of stem, giving a plaited appearance to the shoot.	Variable in size and branching pattern, with red stems 3-12cm tall bearing abundant brown tomentum. Greenish-yellow leaves.	Robust moss often forms extensive pale carpets on bare soil of dry mountain slopes. Plants are pale yellowish-green to brown (moist), white (dry). Stems up to 10cm long.
Habitat	On ground and rotting logs in forest, as epiphyte on bark, occasionally as an epiphyll	Damp, shaded soil in forest	On variety of substrates in forest	In open grassland and roadside banks, as well as in forest.	Damp rocks in the open and in swampy ground, but able to withstand dry periods	Exposed rock and soil, usually on mountains, but also at low altitudes, e.g. on the bare lava of Rangitoto Island, Auckland
Distribution	Widespread in New Zealand; Southern Hemisphere	Throughout New Zealand, probably endemic to New Zealand	Throughout New Zealand; Southern Hemisphere	Throughout New Zealand, worldwide except for the Tropics	Widespread in New Zealand and Australia	Widespread in New Zealand; Southern Hemisphere

2.3 Moisture, Ash Content and Initial pH

The moisture content of the air-dried (AD) samples and the ash (mineral) content was established. All results were recorded on an ash free or oven dry basis.

Approximately 0.5g of air-dried (AD) sample was weighed (in duplicate) in a tared crucible, dried at 105°C for 24hrs, cooled in a desiccator and reweighed giving the oven dry (OD) weight. The sample was then heated in a muffle furnace for 4hrs at approximately 570°C. After cooling this was reweighed to give the ash weight.

Initial pH readings were taken for each sample (n=2). A suspension, with a ratio of 1:2 (sample mass to double distilled water, DDW), was shaken for 30 seconds and the pH read with a Digi-sense pH meter.

2.4 Total Nitrogen determination

Total nitrogen was determined by the Kjeldahl method for total nitrogen as described by (Bremner, 1965a). Samples (n=3) of moss approximately 40-50mg (AD), were digested using mercury, copper or titanium catalyst tablets and concentrated H_2SO_4 for approximately 2 hours. The flasks were cooled on completion and then steam distilled with 10M sodium hydroxide-thiosulphate and the ammonia released collected in boric acid indicator solution. The boric acid solutions were then titrated against standard (0.025M) H_2SO_4 until the end point was reached indicated by the colour change from aqua to pink. The following equation (Equation 1) was used to calculate the amount of ammonium in each distillate.

Equation 1

$$N = \frac{(titre - blank) \times 70}{1000}$$

N = the ammonium-N detected from the distillate in mg

70 = 1mL of 0.025M H_2SO_4 is required to reach an end point with 70μg of ammonium-N in solution.

The value of N then allows calculation of the nitrogen content of the moss samples by relating the N value to the weight of the sample (Equation 2).

Equation 2

$$\%N = \frac{N \times 100}{g}$$

%N = percentage of nitrogen in the sample

g = weight of sample (OD)

2.5 Total oxidisable carbon

To establish the C:N ratios of the various moss species the total oxidisable carbon content was determined using the Walkley-Black method (Hesse, 1971). 10ml of 0.167mol/L^{-1} Potassium dichromate ($K_2Cr_2O_7$) solution and 20mL of conc. sulphuric acid (H_2SO_4) was rapidly added, with swirling, to a 200mL flask containing 50mg (AD) of sample.

The flasks were then left to stand for 30min on an asbestos pad. 100mL of water and 10mL of conc. *o*-phosphoric acid (H_3PO_4) was then added and the flasks left to cool. Once cool, 0.5mL of 0.16% (w/v) barium diphenylamine sulphonate was added and the solution was titrated against a solution of ammonium iron (II) sulphate until an emerald green endpoint was reached. The procedure was repeated twice for each species.

A figure of 44.4% (Krassig *et al*, 1996) total oxidisable carbon for cellulose was used to calculate a correction factor (*f*). Refer to Equation 3.

Equation 3

$$\% \text{ Oxidisable organic carbon (corrected)} = \frac{(\text{blank} - \text{titre}) \times 0.003 \times 100 \times M \times f}{\text{weight of OD sample (g)}}$$

$M = 0.5$ mol/L^{-1} (concentration of ammonium iron (II) sulphate solution)

f = correction factor

2.6 Phosphorous content

To establish the phosphorus content of the moss samples a modified method of analysis was used (Nicholson, 1984). A known quantity of ground litter was ashed in a muffle furnace for 4 hours at 480°C. When cool, the ash was moistened with a few drops of double distilled water (DDW) and then 5mL of 50% HCl added. The crucible was then heated on a boiling water-bath for 5minutes to digest the ash sample. The digest was filtered into a 50mL volumetric flask using hot DDW and made up to volume. The phosphorus content of the digested sample was then determined by the method described by (Kitson and Mellon, 1944). The procedure was prepared in duplicate for each species.

A standard calibration curve was established by using a standard stock phosphate solution of known phosphorus concentration (500 ppm phosphorus). Nitric-vanadomolybdate reagent was added to the stock solution and diluted with DDW. The absorbance at 465nm was then recorded and the curve established. The moss samples were then digested as above and the absorbance read at 465nm. The calibration curve was then used to calculate the phosphorus content of the moss samples via the following equation:

Equation 4

$$\%P = \frac{P^{ppm} \times 25 \times 50 \times 100}{10 \times m \times 1000000}$$

%P = the percentage of phosphorus in the sample

P^{ppm} = the ppm of phosphorus in the vanadomolybdophosphate solution

m = the OD weight of the sample

2.7 Nitrogen distribution analysis

Nitrogen profiles were established using a modified acid hydrolysis procedure (Bremner, 1965b) 1 g (OD) samples (n=2) were hydrolysed (110°C) with 20mL 6M HCl for 24hours. The hydrolysate was filtered and neutralised to pH 6.5. The insoluble fraction was washed and dried at 105°C for 2 hours. Total nitrogen analyses were performed on the soluble and insoluble fractions as described earlier. Ammonium (NH_4^+-N), hexosamine, direct and indirect amino acid analysis was performed. To detect NH_4^+-N, MgO was added to a 10mL sample, distilled for 2 minutes into boric acid and titrated with standard acid. Hexosamine-N plus NH_4^+-N were detected by adding 11mL phosphate borate buffer (PBB) to 10mL of sample, distilling and titrating. To perform direct amino acid analysis (α-amino acid-N) a 5mL sample and 1mL 0.5M NaOH was heated in a water bath for 30minutes. The flask was removed and allowed to cool. 0.52g citric acid and 0.1g ninhydrin was then added and the flask was heated for a further 10minutes. Once cool the sample was distilled with 0.6mL 10M NaOH and 11mL PBB for 4minutes and the distillate titrated with standard acid. The indirect method (α-amino acid-N, NH_4^+-N and hexosamine-N) involved adding 5mL sample, 0.11g Citric acid and 0.1g ninhydrin to a flask and heating for 10 minutes. Once cool the sample was distilled with 0.2mL 10M NaOH and 11mL PBP and the distillate titrated with standard acid. There was good agreement between the values for amino acids determined by both methods.

2.8 Inoculum

The soil inoculum was prepared from a suspension of 1g soil mixed with 9mL water. The soil was collected from the Canterbury University campus at Ilam. The suspension was left to stand (5minutes) to allow the larger particles to settle and the fraction free from debris was used to inoculate to avoid adding extraneous organic matter to the microcosms. All subsequent experiments were inoculated with 3 drops from a Pasteur pipette (approximately 150µl of inoculum).

2.9 Weight loss

Decomposition microcosms were set up containing 1 g (OD) of sample, moisture (approximately 83% by air-dry weight) and soil inoculum. The microcosms were sealed with plastic to reduce the loss of moisture (Figure 3). The microcosms were incubated at 10°C and 25°C. Controls containing a) sample and b) sample, moisture, chloroform and inoculum plus a rubber bung to seal were also set up. The microcosms were set up in triplicate for each species. Microcosms were destructively sampled after 30, 90 and 180-day incubations.

A second series of decomposition tubes was set up in which the moss material added had been subjected to heating at approximately 95°C-100°C for three days. This treatment was used to kill moss material. Tubes were regularly checked for weight loss by weighing and any losses made good.

The decomposition rate factor (k) was calculated for each species using the equation for a curvilinear model (Olson, 1963). See Equation 5.

Equation 5

$$\ln (X_t/X_o) = -kt$$

X_o = the level of decay at time zero

X_t = the level of decay at time t

t = the time elapsed

ln = natural log

Figure 3 Illustration of the weight loss microcosm

2.10 Nitrogen mobilisation

Nitrogen mobilisation microcosms were set up containing 1g (OD) of ground sample, ignited sand (to aerate the ground moss), moisture (approximately 83% by air-dry weight), inoculum and an acid trap (a bijou containing 1mL 2M H_2SO_4). The microcosms were sealed with plastic and a rubber band. (Figure 4)

Another microcosm procedure was similarly set up but used oven-dried unground samples. As the unground moss material occupied three-quarters of the microcosm a lesser amount of 0.5g was chosen. They were set up for the same time intervals.

Both the ground and unground sets of microcosms were set up in triplicate for each species.

Microcosms were regularly checked for moisture loss by weighing. Any losses were amended by addition of DDW.

Microcosms were destructively sampled after 30, 90 and 180-day incubations at 10°C and 25°C.

At each sampling the pH of each microcosm was read after the addition of 10mL double distilled water (DDW).

To estimate nitrate and ammonia, 30mL potassium chloride (2M KCl) was added and then the microcosms were shaken for 60 minutes or left to stand for 24 hours. 10mL of the KCl solution was steam distilled with 0.1g Devada's alloy powder and 0.1g magnesium oxide (MgO) for 3 minutes and the titre read as for total nitrogen analysis. This gave a combined value for nitrate-N and ammonium-N. A second 10mL sample was analysed for ammonium-N by adding MgO, distilling and reading the titre. After the MgO sample was distilled the flask was removed, Devarda's alloy added, and the sample was distilled again with a new boric acid sample. The Devarda's alloy value indicated the presence/absence of nitrate. This was not always tested for if the

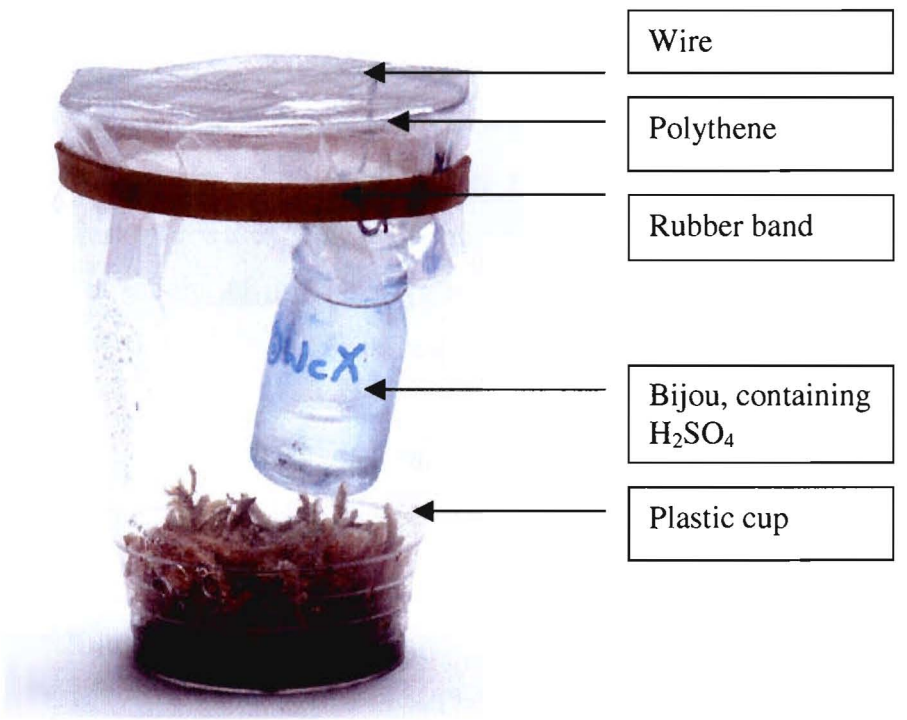
ammonia and the combined values were very similar as this indicated that there was little or no nitrate present.

The acid traps were analysed by transferring the contents of the bijou to a Kjeldahl flask and distilling with sodium hydroxide (NaOH) for 2minutes and then reading the titre.

Initial nitrate and ammonia levels (time zero) were read by setting up microcosms containing sand, sample, water and KCl as above. The microcosms were analysed and these values were used as the control.

An arbitrary value was given to the amount of microbial growth that was visible in the microcosms. This value is based on the percentage of the sample that was covered in microbial growth. A visual description of the colonies was also recorded.

Figure 4 Example of the mineralisation microcosm set up.



2.11 Water-soluble extracts (Leaching)

Weighed amounts, between 0.5g and 0.6, of air-dried ground material were placed in a pre-weighed tube and approximately 20mL DDW was added to each. Chloroform (0.5mL) was also added to prevent microbial growth and the tubes sealed with rubber bungs to prevent chloroform evaporation. The leaching process was set up in duplicate for each species.

After 48hours at room temperature the samples were filtered through GF/A paper via suction filtration. This first filtrate was then used to observe the presence or absence of microbial growth as it contained the highest concentration of leachates. Any solid material that was left on the filter paper was washed back into the tube. The tube was then refilled with DDW and left for a further 3 days when the leachate was removed again. This process was repeated twice more.

After this time the sample was oven-dried to determine weight loss due to leaching. Total nitrogen analysis was also performed, by the Kjeldahl procedure, to establish nitrogen loss during the leaching process.

To establish whether each leachate could support microbial growth the pH was read and then the filtrate was divided into two equal portions and soil inoculum (see 2.8) was added to both. Chloroform was added to one flask to serve as a control. Both flasks were then sealed (Figure 5) and placed on a shaker for 5 days and examined for indications of microbial growth such as turbidity. The pH was also read after the incubation period. The procedure was repeated with non-ground samples.

The nitrogen lost via leaching (Equation 6), the percentage of nitrogen in the leachate (Equation 7) and the mass loss (%) (Equation 8) was calculated for each sample.

Equation 6

$$\text{Nitrogen loss as (\%) of original} = \frac{\text{Mass of N in leachate} \times 100}{\text{Initial mass of N}}$$

Equation 7

$$\text{N soluble (\%)} = \frac{\text{N (g) final} \times 100}{\text{N (g) initial}}$$

N (g) initial = the nitrogen content (g) of the sample before leaching

N (g) final = the nitrogen content (g) of the sample after leaching

Equation 8

$$\text{Mass loss (\%)} = \frac{\text{(Initial organic matter) – (organic matter)} \times 100}{\text{Initial organic matter}}$$

Initial organic matter = Initial sample weight (OD) – initial ash content

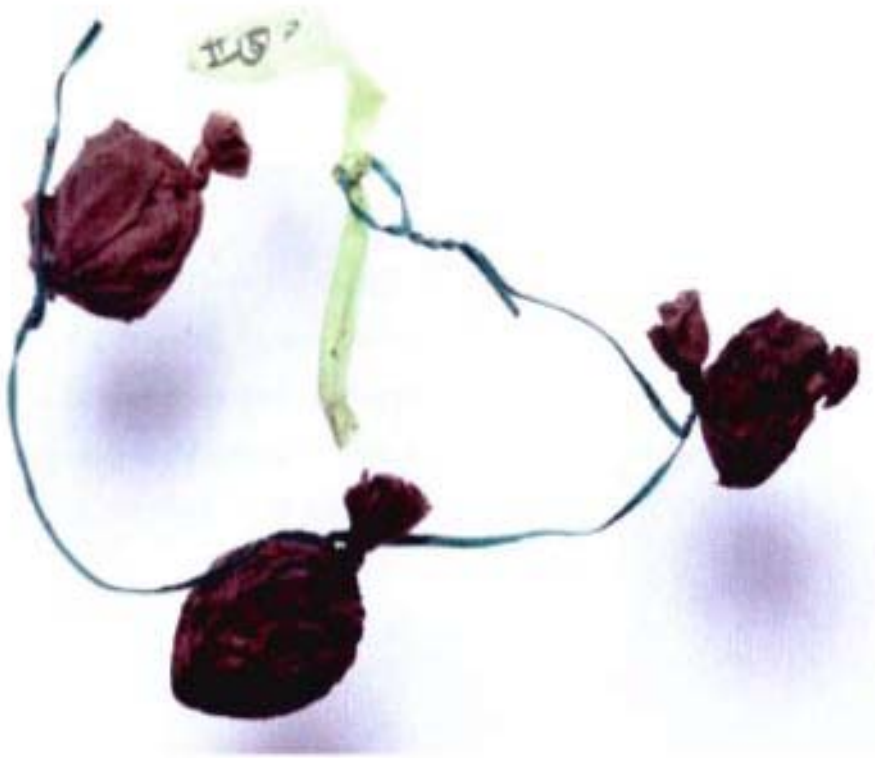
Organic matter = Sample weight (OD) after leaching – ash content

Figure 5 Control (on left) and inoculated sample showing growth during leaching experiment.

2.12 Litterbags

Litterbags were made from stockings mesh size (14-22μ). 0.5-1g of AD whole material was placed in each bag and the bag was tied off. A coloured tag was attached to each bag (Figure 6) before burial 1-2cm below the soil surface in Ilam soil at the Canterbury University campus. The procedure was repeated in triplicate for the following time intervals: 30 and 90 days. After the time period the bags were removed and cleaned of any extraneous debris. The contents was air-dried, oven dried then cooled and weighed. The weight loss was determined and total nitrogen analysis was performed as above. Ash content was also determined for each sample to provide an indication of mineral soil contamination.

Figure 6 Triplicate litterbags



Chapter Three: Results

3.1 Bryophyte characteristics

3.1.1 Oven dry and ash weights

The moisture content of the species after air-drying ranged from 13.4% in *P. aciculare* (brown) to 11.4% in *R. pruinosum* (brown). The moisture content between the brown and green portions of the same species differed in each case ranging from *D. dicarpum*, which had a difference of 0.1% to *H. comatum*, which had 0.54% more moisture in the green portion. Ash values are shown in Table 3.

All weight loss results are recorded on an ash free basis. There appeared to be little evidence of soil mineral contamination but reporting ash free results takes into account into consideration any possible contamination.

3.1.2 Total carbon, phosphorus and nitrogen

Total carbon, phosphorus and nitrogen values for each species are given as a percentage dry weight in Table 3.

Initial total nitrogen (%) ranged from *H. comatum*, which had the largest %N of 1.04 (green), and 1.2 (brown), to the lowest in *R. pruinosum* with 0.6 (green), and 0.36 (brown). There was some variation within species. The same trend was observed for the phosphorus content where *H. comatum* had the largest %P and *R. pruinosum* had the lowest. The percent carbon did not follow this trend.

3.1.3 Carbon, phosphorus and nitrogen ratios

To help in assessing the quality of the moss material in relation to decomposition the C:P and C:N ratios were established for the younger and older regions of each species.

For all species there was a higher C:P ratio than C:N. There was also a notable difference between the C:N and C:P ratios of the green portion of each species in comparison with the brown portion of each species. Table 3

Table 3 Carbon (%), phosphorus (%), nitrogen (%), C:N ratio, C:P ratio and ash content for each species on an oven dry basis.

Sample	C (%)	P (%)	N (%)	C:N ratio	C:P ratio	Ash
Green						
P. aciculare	77 ±0.5	0.26 ±0.01	0.87 ±0.02	89	301	2.7 ±0.02
H. comatum	77 ±1.9	0.36 ±0.02	1.04 ±0.02	74	214	3.1 ±0.01
D. dicarpum	82 ±6.3	0.26 ±0.02	0.82 ±0.03	100	322	3.2 ±0.02
H. cupressiforme	81 ±0.1	0.26 ±0.01	0.67 ±0.05	121	315	2.5 ±0.03
B. pendula	75 ±0.8	0.19 ±0.01	0.70 ±0.01	107	361	3.5 ±0.04
R. pruinosum	81 ±3.9	0.12 ±0.01	0.60 ±0.04	135	640	2.7 ±0.06
Mean	**79**	**0.24**	**0.78**	**104**	**359**	**3.0**
Brown						
P. aciculare	71±2.7	0.18±0.00	0.65 ±0.06	108	394	3.3 ±0.16
H. comatum	63±3.4	0.31±0.00	1.20 ±0.04	53	206	5.5 ±0.02
D. dicarpum	74±0.2	0.22±0.00	0.83 ±0.01	90	339	5.5 ±0.05
H. cupressiforme	77±0.1	0.20±0.00	0.65 ±0.03	118	379	5.7 ±0.02
B. pendula	73±0.1	0.18±0.00	0.77 ±0.05	94	408	5.8 ±0.24
R. pruinosum	67±0.7	0.08±0.00	0.36 ±0.01	188	815	4.5 ±0.57
Mean	**71**	**0.20**	**0.74**	**109**	**424**	**5.1**

Note: Error values given are standard errors of the mean

3.2 Nitrogen distribution

Nitrogen distribution profiles for each type of litter are given in Table 4. Ammonia (NH_4-N), hexosamine (Hex-N), amino acids (α-amino-N), total soluble nitrogen, total insoluble nitrogen and hydrolysable unidentifiable nitrogen were determined.

The insoluble nitrogen from brown *H. cupressiforme* was the highest observed. The hydrolysable unidentifiable nitrogen also had the highest value for brown *H. cupressiforme* and the lowest recording was for green *P. aciculare*.

There is a notable difference between the ammonia-N mean values for the green and brown portions. The mean values for hexosamine-N, amino acids, total soluble nitrogen, total insoluble nitrogen and hydrolysable unidentifiable nitrogen did not vary greatly between the green and brown fractions.

Table 4 Nitrogen distribution analysis of moss species (green and brown forms) used in the study. Values as % of total sample nitrogen

Sample	NH_4-N (%)	Hex-N (%)	α-amino-N (%)	Soluble-N (%)	Insoluble-N (%)	Hydrolysable Unidentified-N (%)
Green						
P. aciculare	9.0±0.05	0.2±0.04	47.4±0.35	89.6±0.01	10.4±0.01	37.6±0.30
H. comatum	6.4±0.07	3.0±1.01	45.2±0.51	88.5±0.01	11.5±0.01	49.8±0.25
D. dicarpum	7.0±0.09	1.1±0.35	43.8±0.54	85.3±0.02	14.7±0.01	47.6±0.20
H. cupressiforme	5.0±0.04	2.3±0.44	42.4±1.08	87.9±0.02	12.1±1.03	50.7±0.05
B. pendula	7.1±0.05	1.8±0.50	45.4±0.34	89.1±0.01	10.9±0.01	47.0±0.15
R. pruinosum	6.5±0.06	2.9±0.53	48.9±0.71	89.6±0.02	10.4±0.02	45.4±1.19
Mean	**6.8**	**2.0**	**45.5**	**88.3**	**11.7**	**46.4**
Brown						
P. aciculare	11.3±0.09	1.2±0.13	44.4±0.02	90.2±0.03	9.8±0.02	43.6±0.12
H. comatum	10.8±0.07	2.1±0.04	39.6±0.05	89.6±0.01	10.5±0.00	46.8±0.12
D. dicarpum	10.6±0.07	1.1±0.10	45.9±1.0	88.0±0.02	12.0±0.02	40.5±0.93
H. cupressiforme	7.6±0.04	3.2±0.17	42.8±0.15	74.7±0.12	25.3±0.12	52.8±0.11
B. pendula	10.3±0.09	1.9±0.02	48.1±0.02	87.5±0.01	12.5±0.01	46.9±0.11
R. pruinosum	10.4±0.07	2.7±0.00	44.6±0.11	88.4±0.01	11.6±0.01	45.0±0.04
Mean	**10.2**	**2.0**	**44.2**	**86.4**	**13.6**	**45.9**

3.3 Water-soluble (leaching)

Leaching experiments were carried out on both ground and whole material. Mass loss was recorded on an oven dry, ash free basis. The results are given in Table 5.

The percentage of mass lost for the whole (unground) samples ranged from 0.7-12.1% across all the species. By comparison, mass loss for ground samples ranged from 1.2-11.0%. The percentages changed from the whole samples to the ground samples with a general increase from whole to ground.

The total nitrogen present in each sample after leaching ranged from 0.3-1.1% for all species including both the ground and whole results. There was not a large difference between the total nitrogen values for each species under both treatments.

The nitrogen mass loss (%) for both treatments over all the species was almost identical. The ground samples ranged from 2.6-5.9% and unground (whole) samples ranged from 2.6-6.1%.

The initial pH was taken before inoculation and incubation and the final pH was read once the incubation period had expired. Generally there was a shift in the pH of the sample during water extraction from being initially acidic (pH 4-5) to basic, falling between 7.1 and 8.2. There were some exceptions where the final pH was either bordering on being basic such as *D. dicarpum* (brown) with a pH of 6.7 or the pH only changed slightly and was therefore still acidic such as *H. comatum* (green) with a final pH of 5.7. The pH readings and level of growth are shown in Table 6.

The majority of the initial leachates were clear or yellow in colour and this did not alter markedly during incubation.

Microbial growth (such as turbidity, fungal flocs and growth on the flask) was observed in all the leachates. The control solutions exhibited zero growth.

Table 5 Amounts of sample mass initial nitrogen content and nitrogen soluble in water as a % of the initial amounts present.

		Whole			Ground		
	Initial Nitrogen (%)	**Mass loss (%)**	**N loss as (%) of initial N***	**N soluble (%)#**	**Mass loss (%)**	**N loss as (%) of initial N**	**N soluble (%)**
Green							
P. aciculare	0.87±0.02	6.5±0.01	0.64±0.01	71±1.0	9.0±0.03	0.61±0.02	69±0.5
H. comatum	1.04±0.02	7.1±0.03	1.05±0.02	97±0.5	9.9±0.02	0.81±0.03	75±0.5
D. dicarpum	0.82±0.03	5.6±0.04	0.62±0.02	74±0.3	6.1±0.03	0.76±0.02	90±0.5
H. cupressiforme	0.67±0.05	12.1±0.01	0.61±0.02	89±0.3	10.4±0.02	0.58±0.04	84±1.0
B. pendula	0.70±0.01	9.7±0.01	0.44±0.02	60±0.5	11.0±0.04	0.50±0.02	70±0.3
R. pruinosum	0.60±0.04	3.7±0.02	0.33±0.03	53±0.3	3.5±0.01	0.41±0.04	67±0.5
Mean	**0.78**	**7.5**	**0.62**	**74**	**8.3**	**0.61**	**76**
Brown							
P. aciculare	0.65±0.06	1.6±0.01	0.74±0.01	109±0.5	4.4±0.02	0.51±0.03	76±0.3
H. comatum	1.20±0.04	10.4±0.03	1.02±0.01	80±0.5	7.9±0.02	0.97±0.04	76±0.5
D. dicarpum	0.83±0.01	3.2±0.03	0.63±0.02	72±0.3	4.0±0.03	0.71±0.03	81±1.0
H. cupressiforme	0.65±0.03	8.1±0.02	0.83±0.02	124±0.5	6.5±0.05	0.62±0.03	93±0.5
B. pendula	0.77±0.05	7.2±0.03	0.65±0.01	78±0.5	5.9±0.03	0.65±0.02	79±0.5
R. pruinosum	0.36±0.01	0.7±0.03	0.34±0.01	90±1.0	1.2±0.03	0.34±0.01	89±0.5
Mean	**0.74**	**5.2**	**0.70**	**80∞**	**5.0**	**0.63**	**82**

* The nitrogen loss as (%) of initial nitrogen is the percent of nitrogen left in the sample after the leaching process

∞ Mean excludes high values of 109 and 124 as they indicate an increase in nitrogen.

The nitrogen soluble (%) is the percentage of nitrogen in the leachate

Table 6 Growth, solution colour, initial and final pH in leaching experiment.

	Whole samples			Ground samples		
	Microbial growth	**Initial pH**	**Final pH**	**Microbial growth**	**Initial pH**	**Final pH**
Green						
P. aciculare	Turbidity fungal flocs	5.7±0.05	7.8±0.05	Turbidity	4.8±0.05	7.8±0.05
H. comatum	Turbidity fungal flocs	5.3±0.10	5.7±0.10	Turbidity	5.3±0.10	7.1±0.10
D. dicarpum	Fungal flocs	5.7±0.10	8.0±0.05	Turbidity	5.0±0.05	7.9±0.05
H. cupressiforme	Turbidity fungal flocs	5.3±0.05	8.2±0.05	Turbidity fungal flocs	4.5±0.05	7.5±0.05
B. pendula	Turbidity	5.8±0.05	8.1±0.10	Turbidity	5.1±0.05	6.9±0.05
R. pruinosum	Fungal flocs	5.5±0.05	7.5±0.10	Turbidity	4.1±0.10	7.8±0.05
Brown						
P. aciculare	Turbidity fungal flocs	5.2±0.05	7.6±0.05	Turbidity	4.6±0.05	7.1±0.10
H. comatum	Fungal flocs	4.9±0.10	7.4±0.05	Turbidity	5.0±0.05	7.3±0.10
D. dicarpum	Fungal flocs	5.5±0.00	6.7±0.10	Turbidity growth on flask	5.0±0.00	5.6±0.05
H. cupressiforme	Turbidity fungal flocs	5.1±0.05	7.9±0.05	Turbidity fungal flocs	4.5±0.10	6.5±0.10
B. pendula	Turbidity	5.4±0.10	7.9±0.10	Turbidity fungal flocs	4.7±0.10	7.2±0.00
R. pruinosum	Fungal flocs	5.4±0.05	6.5±0.05	Turbidity	4.2±0.05	7.2±0.10

3.4 Mass loss due to microbial activity in microcosms

Weight loss is given as a percentage of the mass lost over time (Figure 7-8). There was a general trend at both temperatures of an increase in decomposition over time. *D. dicarpum* (green and brown) exhibited the greatest mass loss at both temperature treatments. Negative weight loss was observed for *R. pruinosum* (green).

Microbial growth was represented as a visual estimate of the percentage cover. The percentage of microbial growth was given an arbitrary value; see Table 7. The microbial growth observed generally decreased over time for all treatments (Table 8).

Refer to Table A.6 (in appendices) for weight loss (%) after 180 days incubation of *H. comatum* and *H. cupressiforme* litter.

Table 7 Arbitrary growth values as a percentage of microbial growth.

Arbitrary value	Details of growth
0	No visible growth
+	Slight signs of growth, > 25% of substrate colonised
++	Growth present, > 25% of substrate colonised
+++	Obvious growth, ≥ 50% substrate colonised
++++	Dense growth, > 60% of substrate colonised
+++++	Dense growth, > 80% of substrate colonised

Table 8 Average microbial growth in the weight loss microcosms at 10°C and 25°C over the 180 day study period.

	10°C			25°C		
Sample	**30**	**90**	**180**	**30**	**90**	**180**
Green						
P. aciculare	+++	++	++	++++	+	0
D. dicarpum	+++	++	0	+++	++	0
B. pendula	+++	++++	0	+++	++	0
R. pruinosum	+++	+	0	+++	+++	+
Brown						
P. aciculare	++	++	++	+++	+	++
D. dicarpum	+++	++	0	+++	++	0
B. pendula	++++	++	+	++	++	0
R. pruinosum	+++	+	0	+++	+++	+

Figure 7 Mass loss of moss litter (whole, green and brown) from microcosms at 10°C

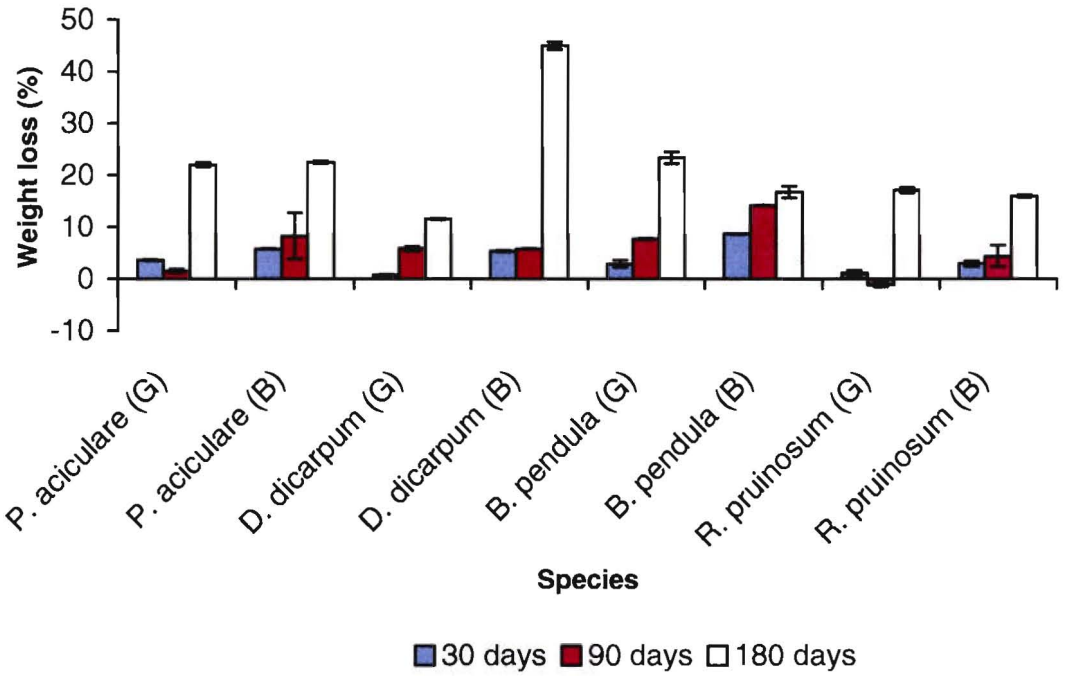
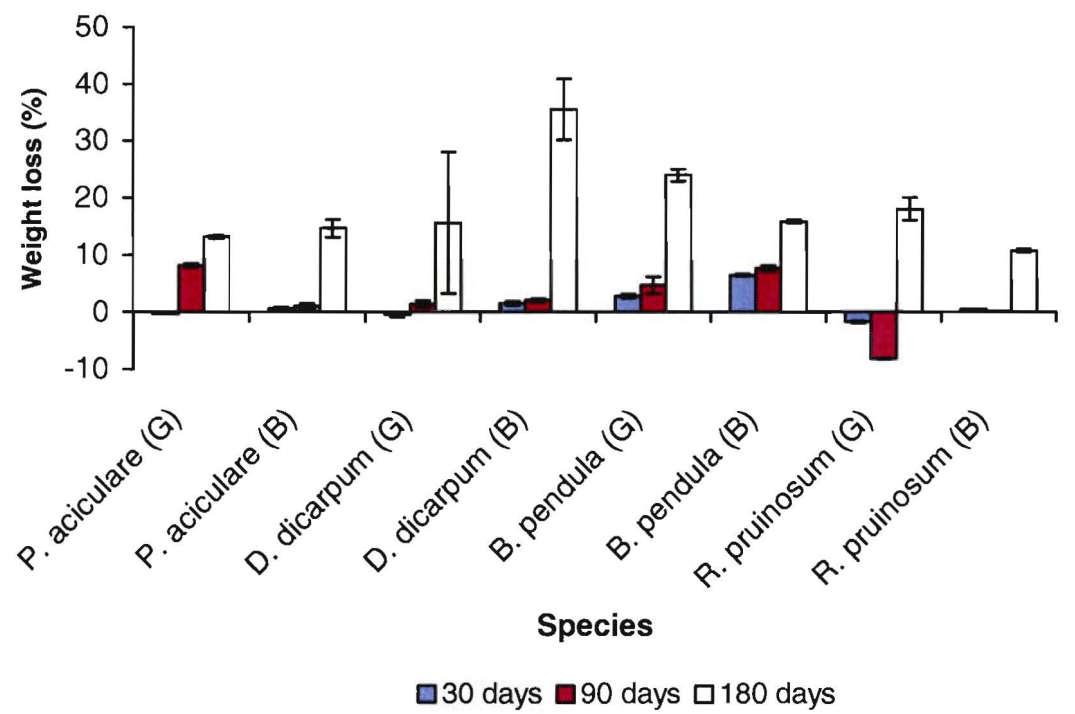


Figure 8 Mass loss of moss litter (whole, green and brown) from microcosms at 25°C



Note: Error values given are standard errors of the mean

3.5 Mineralisation of Nitrogen

3.5.1 Total nitrogen mineralisation

The initial mineral nitrogen of each litter type at time zero and initial pH are shown in Table 9. All the litter types contained negligible mineral nitrogen and were initially acidic.

The average pH for each whole sample at both temperature treatments is shown in Table A.2. There was a general increase in pH during decomposition.

The average pH for each sample (ground) at both 10°C and 25°C is shown in Table A.2.1. There was a general increase in pH during decomposition.

Table 9 Mineral nitrogen (% of initial substrate nitrogen) and pH for each species at time zero.

Sample	Initial Min N (%)	Initial pH
Green		
P. aciculare	0.01±0.01	4.8±0.05
H. comatum	0.04±0.01	4.8±0.10
D. dicarpum	0.04±0.02	4.6±0.05
H. cupressiforme	0.03±0.01	4.5±0.10
B. pendula	0.14±0.01	4.5±0.10
R. pruinosum	0.01±0.01	4.3±0.10
Brown		
P. aciculare	0.07±0.01	4.7±0.05
H. comatum	0.02±0.01	5.0±0.10
D. dicarpum	0.04±0.01	4.6±0.10
H. cupressiforme	0.06±0.01	4.5±0.05
B. pendula	0.15±0.01	4.7±0.05
R. pruinosum	0.02±0.02	5.5±0.10

The average microbial growth for each ground sample was recorded and is shown in Table A.3. Generally there was a decrease in microbial growth over time.

The average microbial growth for each triplicate (whole) was recorded. Generally there was a decrease in microbial growth with time. Refer to Table A.3.1.

Refer Table A.5 for total mineralised nitrogen (%) for whole *H. comatum* and *H. cupressiforme* litter after 180 days incubation.

P. aciculare and *D. dicarpum* showed a general increase in percent nitrogen mineralised for both green and brown litter (whole) at 10°C. *R. pruinosum* exhibited a peak at 90 days for both green and brown portions and *B. pendula* brown litter generally increased but green litter had a slight peak at 30 days (Figures 9 and 10).

The similar trends were seen for the litter (whole) at 25°C. The range of percentages mineralised for each litter sample, however, was not identical between the temperatures. See Figures 11 and 12.

Ground (green) *R. pruinosum* litter exhibited the same trend as the unground litter at 10°C, but the brown samples lacked the peak at 90 days. *P.aciculare* and *D dicarpum* both exhibited peaks at 30 days for both litter types. *B. pendula* exhibited peaks at 90 days for both litter types (Figures 13 and 14).

The similar trends were exhibited by ground litter samples at 25°C. There was a difference in the range of nitrogen mineralised for each litter sample between the temperatures (Figures 15 and 16).

Figure 9 Mineralisation of the nitrogen in whole, green moss litter at 10°C

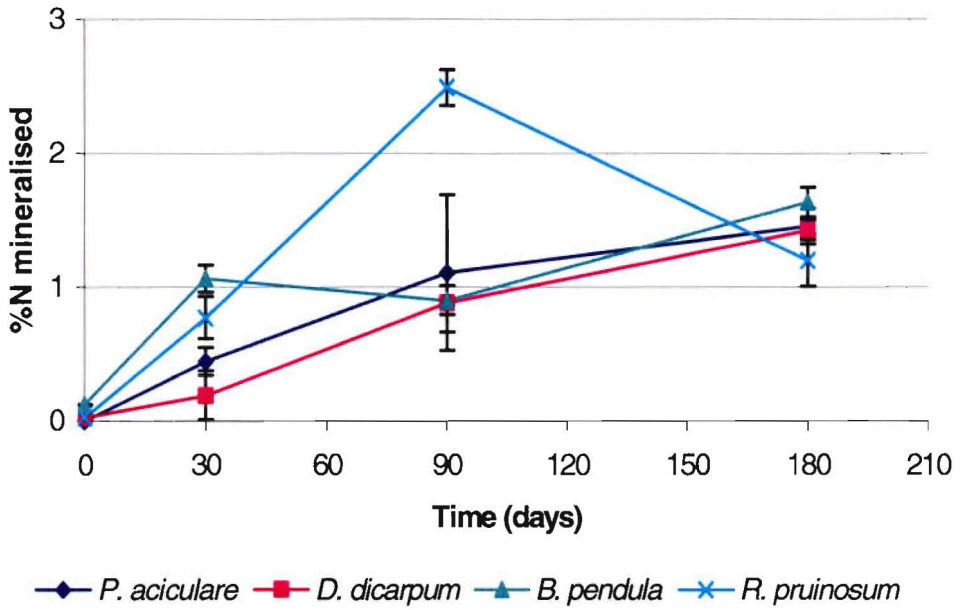
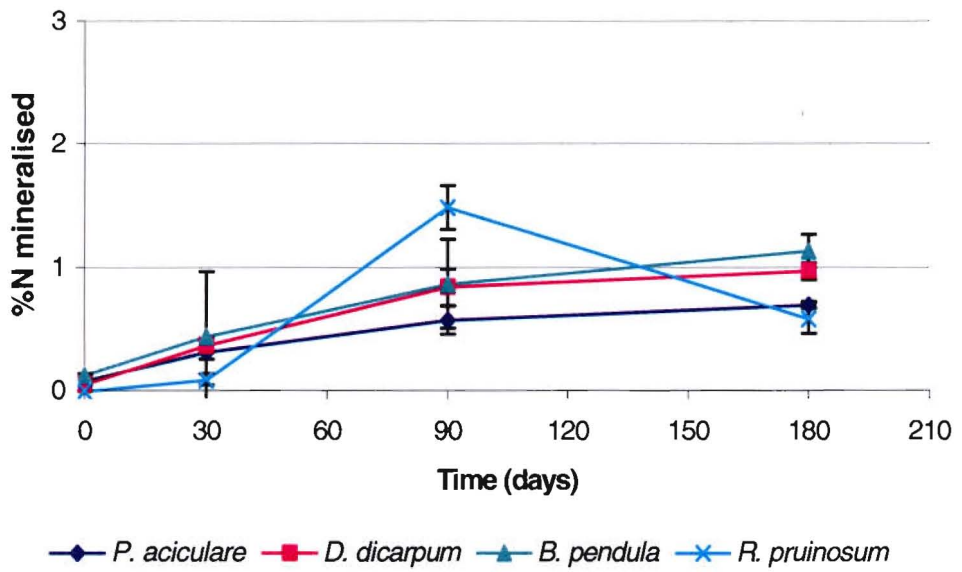


Figure 10 Mineralisation of the nitrogen in whole, brown moss litter at 10°C



Note: Error values given are standard errors of the mean

Note: %N mineralised is a percentage of the initial sample nitrogen.

Figure 11 Mineralisation of the nitrogen in whole, green moss litter at 25°C

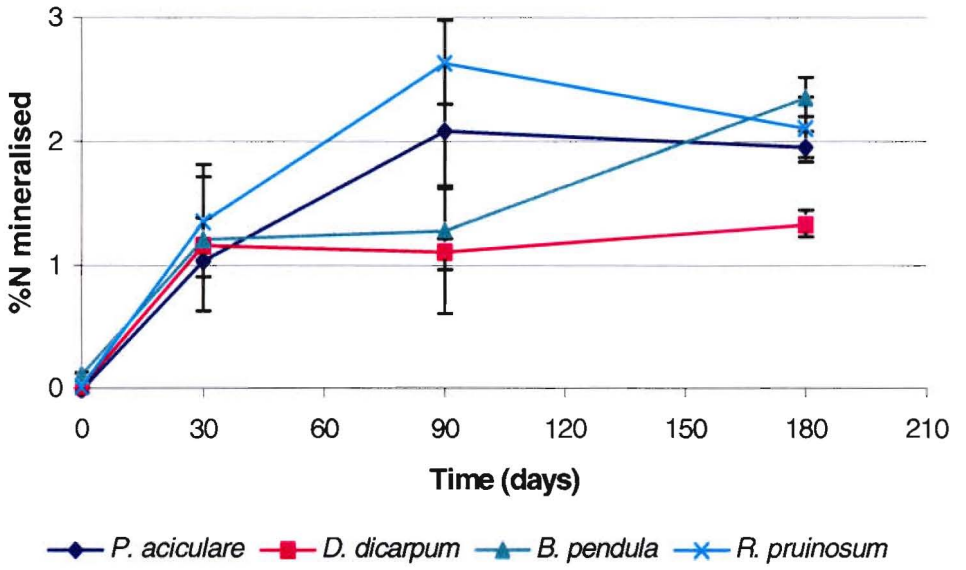
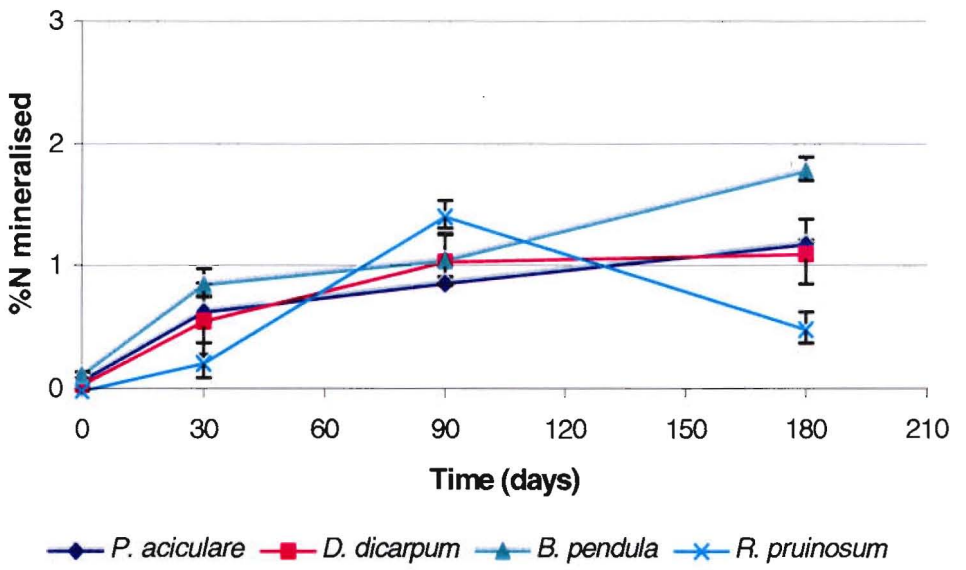


Figure 12 Mineralisation of the nitrogen in whole, brown moss litter at 25°C



Note: Error values given are standard errors of the mean

Figure 13 Mineralisation of the nitrogen in ground, green moss litter at 10°C

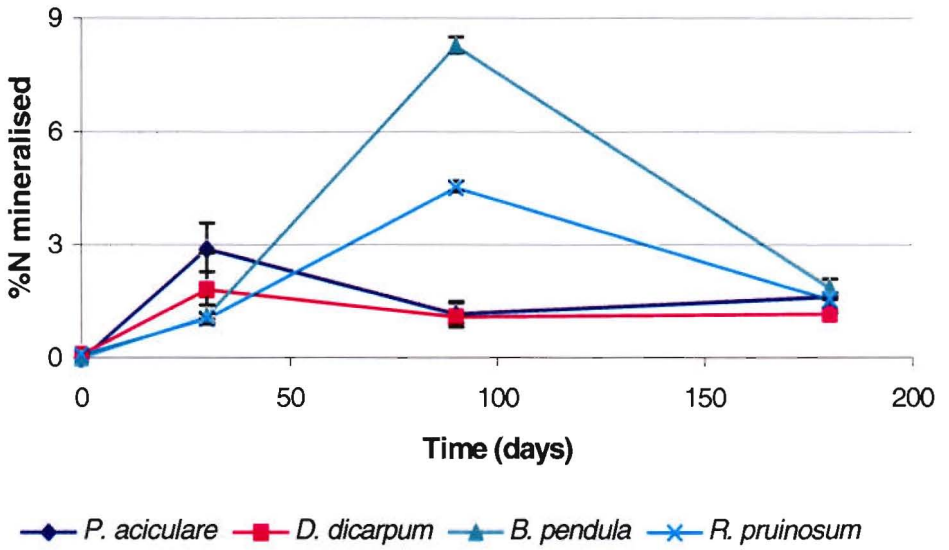
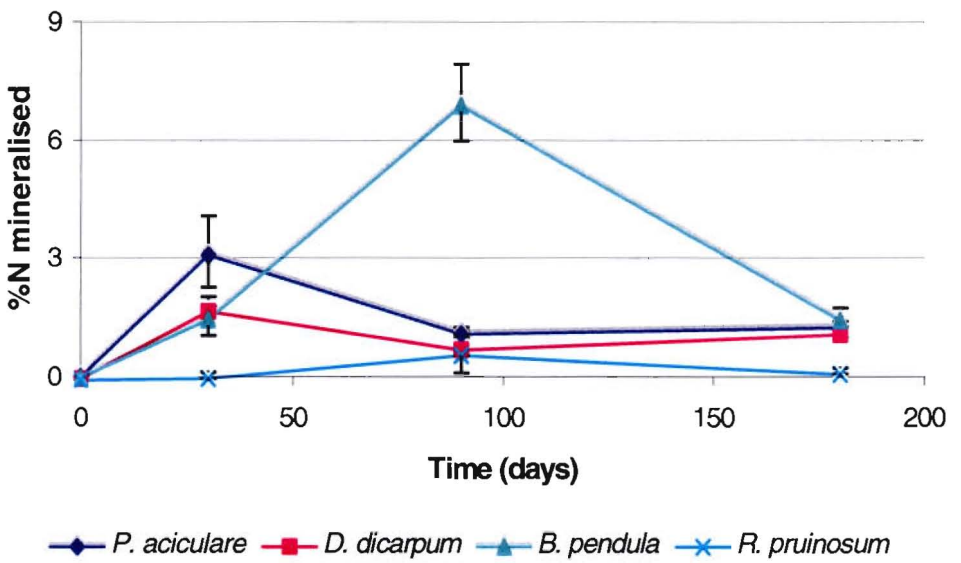


Figure 14 Mineralisation of the nitrogen in ground, brown moss litter at 10°C



Note: Error values given are standard errors of the mean

Figure 15 Mineralisation of the nitrogen in ground, green moss litter at 25°C

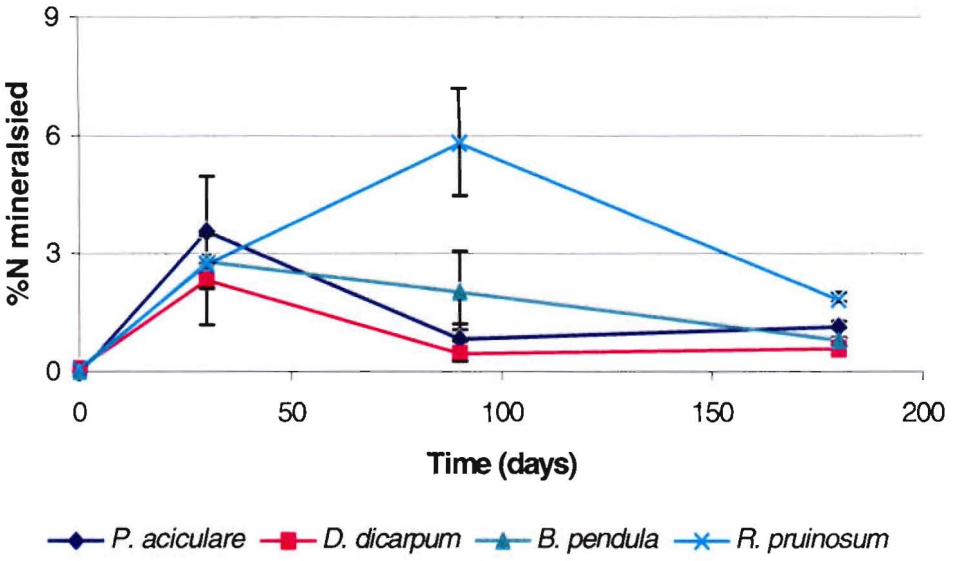
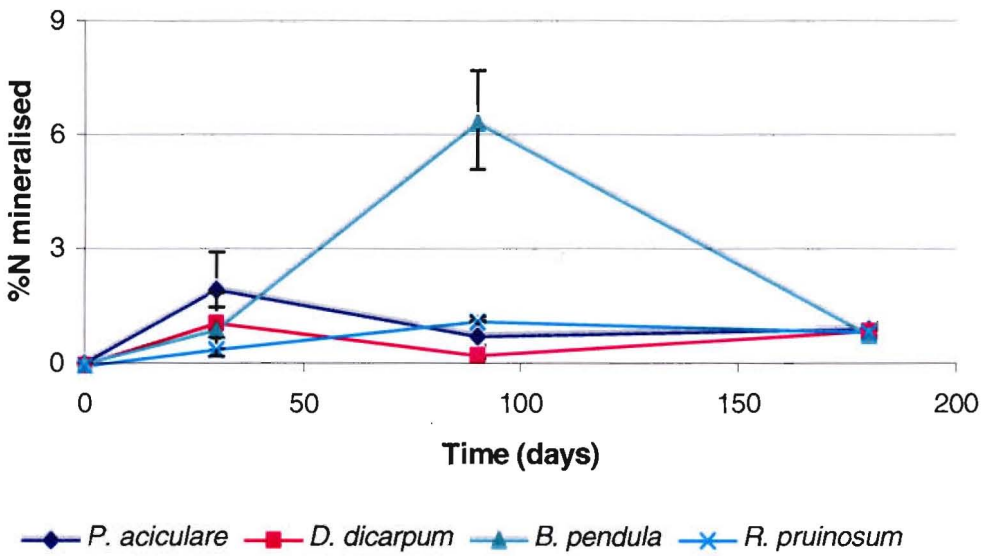


Figure 16 Mineralisation of the nitrogen in ground, brown moss litter at 25°C



Note: Error values given are standard errors of the mean

3.5.2 Nitrate levels detected in the mineralisation microcosms

Generally there was little or no nitrate present in the microcosms. Green and brown litter of *P. aciculare, H. comatum, D. dicarpum, B. pendula* and *R. pruinosum* (green only) all had noticeable nitrate levels after 30 days at 10°C. See Table 10. The nitrate levels detected at 25°C for ground litter were generally higher for each time period and for each litter type than the equivalent 10°C sample. See Table 10.

The nitrate values for whole litter at both temperature treatments are shown in Table 11. The results are generally lower for whole litter than for the ground samples at 10°C. Whereas at 25°C the reverse trend occurred with the higher nitrate levels at 180 days.

Table 10 Nitrate (% of total substrate nitrogen) for ground litter at 10°C and 25°C.

Ground	**10°C**			**25°C**		
	30 days	**90 days**	**180 days**	**30 days**	**90 days**	**180 days**
Green						
P. aciculare	2.9	0.4	-0.1	3.6	0.9	-0.1
H. comatum	3.4	0.1	-0.1	2.9	0.4	0.8
D. dicarpum	2.8	0.0	-0.1	3.6	0.0	-0.1
H. cupressiforme	0.1	ND	-0.1	2.4	ND	-0.1
B. pendula	1.2	-0.4	0.0	2.9	1.2	0.0
R. pruinosum	1.1	-0.1	-0.1	2.8	0.9	0.1
Mean	**1.9**	**0.0**	**-0.1**	**3.0**	**0.7**	**0.1**
Brown						
P. aciculare	3.2	0.3	-0.1	2.0	0.8	-0.1
H. comatum	1.5	0.2	-0.1	2.7	0.2	0.8
D. dicarpum	1.7	0.0	-0.1	1.1	0.2	-0.1
H. cupressiforme	0.0	ND	-0.1	0.6	ND	-0.1
B. pendula	1.5	0.4	-0.1	1.4	5.6	0.0
R. pruinosum	0.0	-0.1	-0.1	0.4	-0.2	-0.1
Mean	**1.3**	**0.16**	**-0.1**	**1.4**	**1.3**	**0.1**

Note: ND refers to species that were not tested for logistical reasons.

Table 11 Nitrate (% of total nitrogen mineralised) for whole litter at 10°C and 25°C.

Whole	10°C			25°C		
	30 days	90 days	180 days	30 days	90 days	180 days
Green						
P. aciculare	-0.1	0.0	-0.1	0.3	1.0	1.6
H. comatum	ND	ND	-0.1	ND	ND	1.1
D. dicarpum	-0.1	-0.1	-0.1	1.2	0.6	1.2
H. cupressiforme	ND	ND	0.1	ND	ND	3.4
B. pendula	0.0	-0.1	0.0	0.9	1.5	2.1
R. pruinosum	-0.1	-0.1	-0.1	0.0	1.2	1.1
Mean	**-0.1**	**-0.1**	**-0.1**	**0.6**	**1.1**	**1.8**
Brown						
P. aciculare	-0.1	-0.1	-0.1	0.1	0.3	1.1
H. comatum	ND	ND	-0.1	ND	ND	0.8
D. dicarpum	-0.1	-0.1	-0.1	0.6	0.4	0.9
H. cupressiforme	ND	ND	-0.1	ND	ND	3.2
B. pendula	-0.1	-0.1	0.0	0.2	0.5	1.7
R. pruinosum	-0.2	-0.2	-0.1	-0.1	-0.0	0.3
Mean	**-0.1**	**-0.1**	**-0.1**	**0.2**	**0.3**	**2.0**

Note: ND refers to species that were not tested for logistical reasons.

3.6 Litterbags

There were no obvious differences between species exhibited by the litterbag results (Table 12). A small amount of growth was observed in a small number of the bags. No animal interference of the litterbags was observed after burial in the field.

Table 12 Mass loss (%) and nitrogen (%) from litterbags place in the field after 30 and 90 days.

		30 days		90 days	
Sample	**Initial %N**	**Weight loss (%)**	**Nitrogen (%)**	**Weight loss (%)**	**Nitrogen (%)**
Green					
P. aciculare	0.87	5.8	0.60	3.7	0.87
D. dicarpum	0.82	ND	ND	7.1	0.59
B. pendula	0.70	ND	ND	5.1	0.54
R. pruinosum	0.60	2.1	0.40	-1.9	0.41
Brown					
P. aciculare	0.65	-0.1	0.60	0.7	0.75
D. dicarpum	0.83	ND	ND	-1.6	0.75
B. pendula	0.77	ND	ND	3.3	0.66
R. pruinosum	0.36	-0.7	0.40	-3.5	0.31

ND: the species was not studied in the field at 30 days due to logistical reasons.

Chapter Four: Discussion

4.1 Chemical characteristics of the species studied

Litter quality is one of three major factors: 1) the nature of the decomposer community 2) the resource quality and 3) the physico-chemical environment, which are considered to be influential in the decomposition of plant litter (Heal *et al*, 1997). The chemistry of the litter may indicate whether the substrate will be palatable or unpalatable to decomposer organisms and this will greatly influence the rate at which the litter is broken down (Brown and Bates, 1990).

Bryophytes have been frequently indicated to be unsuitable substrates for many organisms due to aspects of their chemistry (Longton, 1984). It has been suggested that they possess higher C:N ratios, larger amounts of holocellulose and crude fibre and lower overall energy levels than vascular plants which therefore makes them less appealing as a food source for herbivores (Russell, 1990). Bryophyte material has also been indicated to have an unpleasant taste, such as bitter or hot, and can also have an odour that may repel herbivores (Asakawa, 1990). These aspects of the plant chemistry could be important in discouraging herbivory (Asakawa, 1990).

"Lignin-like" compounds and other polyphenolic compounds are also thought to be present in some species of moss (Erickson and Miksche, 1974). Lignin and other polyphenolic compounds are considered to be major contributors to regulation of decomposition processes (Swift *et al*, 1979). Polyphenols are known to affect litter quality, and in some cases have a greater effect on decomposition rates than parameters such as nitrogen and lignin (Hattenschuiler and Vitousek, 2000). Erickson and Miksche (1974) tentatively investigated the presence of lignin or other polyphenols in a number of liverworts and mosses. Their findings lead them to tentatively suggest that three types of phenolic cell wall compounds were present in the samples and that none of the species analysed had lignin. Brown and Bates (1990) add support to the suggestion that the lack of herbivory in relation to bryophytes may be due to the presence of phenolic compounds. Gerson (1982) also reviewed

information that indicated that bryophytes produce other toxic secondary substances (such as oxalic acid which repels moth caterpillars and phytophagous mites, and benzyl benzoate which is lethal to insects and mites) and that their relatively nutrient-poor composition may also be important in explaining their lack of appeal to herbivores.

C:N ratios have been used commonly as an indication of litter quality but many studies have suggested that lignin:N ratios are a better predictor of decomposition (Meentemeyer, 1978; Keenan *et al*, 1996). Lignin or "lignin-like" compounds were not determined for the moss species in this study, therefore the lignin:N ratio was not calculated. Taylor *et al* (1989), however, concluded after comparison of lignin:N and C:N ratios in microcosms that C:N ratios provided the most accurate predictive power for decomposition in microcosm studies.

The total nitrogen content (%) established during chemical analysis of each species agreed with the range of percentages stated for moss species in previous research (Coulson and Butterfield, 1978; Prins, 1982; Berg, 1984; Hobbie, 1996). However, no direct comparison could be made for the nutrient contents of the moss material with past work as no literature data could be found on the species selected for this study. *H. comatum* exhibited the greatest nitrogen content for both the green and brown fractions whereas *R. pruinosum* had the lowest. The phosphorus content followed the same trend as the %N and agreed with trends in past work on other moss species (Coulson and Butterfield, 1978; Prins, 1982; Smith and Walton, 1986). Carbon percentages were considerably greater than both the nitrogen and phosphorus values. These results again agreed with earlier work (Hobbie, 1996) and allowed calculation of C:N and C:P ratios. The high C:N and C:P ratios (for both green and brown) indicate that moss litter is of low quality (Killham, 1994) and therefore is probably more resistant to breakdown. Comparison of the means for carbon, phosphorus, nitrogen, C:N and C:P ratios indicated that there was not a large difference between the green and brown fractions for any of the six species. The litter quality in relation to these parameters did not appear to vary greatly between the green and brown fractions.

The nitrogen distribution data indicated that α-amino-N constituted approximately 40-50% of the acid soluble nitrogen for both the brown and green litter. Approximately 46% of the nitrogen released via hydrolysis (for both green and brown) was hydrolysable unidentifiable-N. Greenfield (1992) suggested that the majority of the hydrolysable unidentifiable-N fraction consisted of non α-amino acids and imino acids (e.g. proline). The NH_4-N is probably derived largely from amides (Greenfield, 1999). Addition of the NH_4-N, α-amino-N and HUN percentages indicates that approximately 90% of the hydrolysate nitrogen is derived from amino acids, probably in proteinaceous compounds. This could increase the quality of the litter, but if the amino acids and proteins are protected by complexing with other compounds then the litter quality could be reduced. The hexosamine values were probably from microbial origin. To establish whether there was statistically any significant difference between the means of the green and brown material a t-test was performed on the data for NH_4-N, α-amino-N, soluble-N, insoluble-N, and hydrolysable unidentified-N. The results indicated that there was no significant difference between the means, therefore the green and brown fractions were not significantly different.

Litter quality alters over time due to degradation of easily metabolisable substrates and accumulation of recalcitrant compounds. During the breakdown of the litter compounds can complex and form more resistant forms, for example polyphenols can complex with proteins thereby producing a complex which is considered to be highly resistant to microbial attack (Hattenschuiler and Vitousek, 2000).

Berg and Staaf (1981) studied the release of nitrogen in relation to the phases of release, which occur during the decomposition of litter material. The following three phases have been distinguished which indicate the pattern of nitrogen release in a decomposing system: Leaching phase, accumulation phase and final release phase.

Leaching phase constitutes a rapid release of initially leachable nitrogen in litter. It is short and runs from the start of weight loss until the end of initial net release of nitrogen. In some cases leaching is not distinguished from the accumulation phase.

Accumulation phase (or immobilisation) begins at the point where net absolute increase in nitrogen takes place after a leaching phase or from the start of weight loss without leaching. This phase ends when a maximum in the absolute amount of nitrogen is reached.

The final release phase involves a net release after a maximum amount of nitrogen has been accumulated in litter.

4.2 Leaching

The water-soluble organic matter component of litter which comprises largely organic acids, amino acid and inorganic ions provides a readily available energy source for decomposers and has, therefore, the greatest influence on the initial stages of decomposition (Williams and Gray, 1974). The extracts contain physiologically active compounds, which have varied effects on organisms in the soil environment (Nykvist, 1959). It has been indicated that there is a positive correlation between the amount of water-soluble substances in the litter and its decomposition during the first months. This phenomenon is probably due to the water-soluble compounds being leached out of the litter and either easily decomposed by the soil microorganisms or lost in the soil solution (Nykvist, 1959; Berg and Staaf, 1981).

In this study the percentage of soluble nitrogen was high and ranged from 53%-97% for both whole and ground litter. The action of grinding in some cases increased the amount of nitrogen lost, but generally grinding did not appear to have a great influence on the quantity of nitrogen removed. The mass that was lost during the extraction process generally increased from unground to ground samples. This suggests that although ground samples resulted in a greater mass loss, grinding did not alter the amount of soluble nitrogen. The additional mass is probably due to loss of other substances that were released to the water extraction solution via the grinding action. The other substances could be sugars, organic acids and possibly polyphenols. Nykvist (1959) also found that there was a greater loss of water-soluble compounds from ground samples than unground litter.

The mass of nitrogen lost during the extraction process was generally greater for the older (brown) regions of both the ground and whole litter. Older regions contain dead and senescent material in which the cells are dead or dying. These cells are more susceptible to attack by microbes due to alterations that occur during aging of plant material. Aging or senescence of plant material involves the slowing of metabolic activity, reduction in apical and cambial growth, smaller leaves, reduced production of flowers and seeds, and a greater susceptibility to parasitic attack and abiotic stress (Larcher, 1995). Aging cells exhibit distinct changes such as breakdown of chlorophyll in the chloroplasts, disappearance of the thylakoid membranes, shrinkage of the cytoplasm, an increase in catalytic enzymes (for example proteases, hydrolases and peroxidases) and an increase in permeability of the biomembranes to ions, soluble carbohydrates and amino acids (Larcher, 1995). The breakdown of the protein within the cells results in an increase in the quantities of soluble amino compounds (Burgess, 1985), which can be transported to regions such as seeds and younger parts of the shoot for reuse (Larcher, 1995). This translocation of protein breakdown products also facilitates the recycling of important elements such as nitrogen, phosphorus and sulphur (Larcher, 1995) The loss of metabolic activity (such as production of inhibitory secondary metabolites) and structural integrity within the senescent cells indicates the vulnerability of dead and dying material to microbial breakdown.

Table 14 gives examples of nitrogen leached from various plant litters. In comparison with the results described in Table 13 the percentage of the leached nitrogen lost from the moss litter was generally considerably higher than the figures reported in the papers referred to in Table 14. This suggests that the quantity of nitrogen released by leaching from moss litter is higher than the soluble nitrogen liberated from the litter of higher plants, although quantitatively the nitrogenous substances seem to be similar.

Table 13 Leached nitrogen (% of total litter nitrogen) for all litter types

	Whole	Ground
Sample	**Leached Nitrogen (% of total litter nitrogen)**	**Leached Nitrogen (% of total litter nitrogen)**
Green		
P. aciculare	30	31
H. comatum	2.6	25
D. dicarpum	26	10
H. cupressiforme	11	16
B. pendula	40	30
R. pruinosum	45	33
Brown		
P. aciculare	ND	24
H. comatum	20	24
D. dicarpum	28	19
H. cupressiforme	ND	7
B. pendula	22	21
R. pruinosum	10	11

Note: ND values could not be calculated due to experimental error.

Table 14 Leaching of nitrogen from (whole) needle and leaf litter

Species	Litter type	Total nitrogen of litter (%)	Leached nitrogen (% of total litter nitrogen)	Reference
Ash (*Fraxinus excelsior*)	leaves	1.1	15	(Nykvist, 1959)
Hazel (*Corylus avellana*)	leaves	2.1	10	(Howard and Howard, 1974)
Oak (*Quercus petraea* and *(Q. robur*)	leaves	0.37	10	(Howard and Howard, 1974)
Willow (*Salix sp.*)	leaves	1.0	10	(Hodkinson, 1975)
Beech (*Fagus grandifolia*)	leaves	0.85	25	(Gosz *et al*, 1973)
Scots pine (*P. silvestris*)	needles	0.38	10	(Staaf and Berg, 1977)

The extent of leaching in the laboratory experiment would be expected to be greater than in the field as the litter in the field would probably not be exposed to the extensive extraction regime employed in the laboratory experiment.

Allelopathic qualities are suggested to be factors influencing the breakdown of bryophyte material (Rice, 1984). Allelopathy refers to the "direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment" (Rice, 1984). The influence of allelopathic compounds is debatable and has been suggested to be unimportant at a population level and is suggested to be more effectively applied to the ecosystem level (Wardle *et al*, 1998).

Bryophytes have been acknowledged as having compounds with medicinal properties, which prompted a study of their bioactive substances (Asakawa, 1990). Among the

bioactive compounds isolated from bryophyte material were substances with antimicrobial and antifungal activity. These substances were not classed as allelopathic but may still have an effect on microbial colonisation and decomposition (Asakawa, 1990).

All the leachates supported various degrees of microbial growth. The solutions became turbid within 2-3 days of inoculation, and many of them contained fungal flocs. These results indicated that soil microorganisms were able to metabolise the compounds in the leachates and produce a substantial amount of growth. It also suggested that the water-soluble substances were not toxic to all the microorganisms present although it is reasonable to suggest that some microorganisms may have been affected.

The pH for each leachate increased during the incubation period. Nykvist (1959) also found an increase in alkalinity occurred due to utilisation of the compounds in the leachate. The increase in alkalinity is probably due to release of cations (such as Na^+, K^+, Ca^{2+}) and also ammonium, which may help to neutralise acids.

4.3 Weight loss

As decomposition proceeds mass loss occurs and the litter is reduced in size. The processes of leaching, catabolism and physical breakdown (comminution) cause the reduction in mass. The loss of weight is the result of a combination of these processes and so it is used as an indicator of decomposition.

Leachates are usually easily removed from ground but not intact material and would be expected to be utilised in a decomposing system, therefore, it seems reasonable to suggest that the initial loss in weight exhibited by litter is probably caused by this effect (Nykvist, 1959).

The laboratory microcosms exclude any vertebrate or invertebrate action (but may contain microfauna e.g. nematodes) and so any decomposition occurring (excluding leaching) is largely due to microbial activity.

The weight loss results exhibited rates and values that were similar to previous work (Clymo, 1965; Berg, 1984; Farrish and Grigal, 1984; Smith and Walton, 1986; Van Tooren *et al*, 1987; Van Tooren, 1988; Russell, 1990; Johnson and Damman, 1991; Hogg *et al*, 1994), although it has to be noted that the literature data was derived from litterbag experiments and not laboratory microcosms therefore direct comparisons cannot be made. Weight loss values are considered to be underestimates, as microbial biomass was not removed prior to weighing of the tubes. An initial decrease in weight of between 0-10% was noted in the first 30 days at both temperatures. The decrease in weight then continued but at a slow rate till after 90 days there was between 0-15% mass loss. In the next 90 days there was an increase in the rate of decomposition in most cases, resulting in the mass loss falling between 10-45%. This suggests that initially there is a loss of easily decomposable compounds such as water-soluble leachates. Once the leachates have been removed there comes a time when the microbial population has to begin attacking the less easily decayed compounds e.g. cell walls. During this time the population is probably limited by nitrogen and phosphorus as the moss plants exhibit high C:N and C:P ratios and fungal and bacterial growth is suggested to be optimal at much lower ratios (e.g. ~25) (Heal *et al*, 1997). As the carbon is released the ratio will alter and eventually the nitrogen and phosphorus will not be limiting and the rate of breakdown will increase (Swift *et al*, 1979).

The increase from 90-180 days was considerable for all the litter types at both temperature treatments, which suggests that the microorganisms had, through enzymatic action, chemically changed the litter thereby making the substrate more readily available for attack. *D. dicarpum* exhibited the greatest mass loss (%) over the 90-180 day period. The considerable increase in loss suggests that the substrate was not limiting microbial growth and that the quality of the litter was relatively high during this period.

There was no obvious influence of temperature on the rate of weight loss after 180 days but there did appear to be a difference at 30 and 90 day samples. For all the species (excluding *P. aciculare*, green) there was an apparent trend towards less weight loss (activity) at 25°C than at 10°C. This suggests that the microorganisms from the inoculum were more accustomed to lower temperatures (such as 10°C) and that they required time to acclimatise to the higher temperature before a higher rate of decomposition activity could occur.

The decay factors (k) for each species were generally below 0.6 after 180 days decomposition (refer to Table 15), which indicates a slow rate of decay. In comparison to other forms of litter (see Table 16 modified from Maclean and Wein, 1978) the k factors are more similar to those shown for leaf decay whereas branch decay is considerably slower which is probably due to the known presence of large amounts of polyphenols, lignin, low nitrogen levels and absence of water-soluble material. A comparison between the literature and experimental results, however, can only be made with caution as the experimental results were derived from laboratory assays and the literature results came from litterbag experiments. It is also possible that the experimental k values are an overestimation as laboratory experiments are undertaken in controlled conditions and so the rate of decomposition may exceed the rate in field conditions.

Table 15 k values for 180 days decomposition at both temperature treatments in microcosms

Sample	k at 10°C	k at 25°C
Green		
P. aciculare	0.51	0.52
H. comatum	0.41	0.60
D. dicarpum	0.25	1.30
H. cupressiforme	0.37	0.26
B. pendula	0.54	0.37
R. pruinosum	0.38	0.35
Brown		
P. aciculare	0.29	0.32
H. comatum	0.42	0.47
D. dicarpum	0.34	0.90
H. cupressiforme	0.37	0.30
B. pendula	0.56	0.35
R. pruinosum	0.40	0.23

Table 16 Decomposition parameters for various types of whole litter in litterbags, buried for 365 days at a temperature range of -13°C to 18°C (Maclean and Wein, 1978)

Litter type	k
Jack pine needles	0.31
Red maple leaves	0.72
Understory litter	0.27
	0.21
	0.25
Forest floor material	0.13
	0.28
	0.20
Jack pine branches	0.08
Red maple branches	0.07

Following an initial burst of microbial growth, the amount of visible growth then generally decreased during the decomposition period for both temperatures. This could be due to the initial release of easily decomposed soluble compounds. The subsequent decrease in growth is probably a result of a decrease in readily decomposable compounds (after utilisation by the microbial population) leaving the more recalcitrant substrates. There was no obvious difference in growth between the green and brown portions. The growth was predominately white hyphal growth, although a grey hyphal network was also present in some cases. Green, cream, white, pink, and black sporulating colonies were noted on many of the microcosms but they did not dominate over the white hyphal growth.

The variation in growth forms and coverage of the litter observed in the microcosms is in keeping with the suggestion of succession occurring on decomposing litter. As the chemistry of the litter changes the microbes capable of attacking the litter also changes which creates a microbial succession (Wardle and Laville, 1997).

Not all the earlier studies into decomposition have quantified the microbial growth or identified the microbial species present as the focus has been on the loss of weight or elements from the litter (Van Tooren *et al*, 1987; Van Tooren, 1988; Rochefort *et al*, 1990; Russell, 1990). The knowledge of the microbial species involved in bryophyte decomposition appears to be limited. Frankland (1974) reviewed work that was focused on the presence of microbes on moss material. The work presented suggested that various fungi (such as Ascomycetes and Basidiomycetes) and bacteria have been identified but whether or not they were involved in the actual decomposition was not clear.

4.4 Mineralisation

Mineralisation is the microbial mediated breakdown of larger organic compounds, such as proteins, into smaller inorganic molecules. This process is important to nutrient cycling because the breakdown products are in a form that can be utilised by plants and incorporated into new biomass (Pleczar Jr *et al*, 1993). Carbon or nitrogen

mineralisation studies have been used in the study of litter decomposition in the past through monitoring CO_2 evolution or ammonium production. However, in the case of bryophytes, mineralisation has not been used as an indicator of decomposition and nutrient flux. The results presented in this research are therefore not comparable to previous work on bryophytes and this indicates the need for further experimental work in this area.

The nitrogen mineralised in the microcosms was generally in the form of ammonium ions (NH_4^+). There were only small amounts of ammonium volatilised and detected in the acid trap, generally less than 0.5% of the total initial nitrogen.

Whole litter (green and brown) at both temperature treatments exhibited a lower percentage nitrogen mineralisation than the equivalent ground samples. This again suggests that grinding breaks down tissues that would otherwise be difficult for the microorganisms to metabolise. Herbivory of moss litter would create ground material via comminution, but as it has been indicated that moss material is not preferentially grazed by organisms other than in cold climates (Prins, 1982) the lack of herbivory probably adds to the slowed mineralisation rate.

R. pruinosum and *B. pendula* exhibited peaks of nitrogen mineralisation at 90 days for some of the treatments. *R. pruinosum* consistently reached a peak after 90 days for all the litter types and temperature treatments (excluding ground, brown litter at 10 and 25°C). In the case of the brown (ground) litter the rate of nitrogen release was almost linear. *B. pendula* material displayed peaks for all ground litter types at both temperatures except green moss at 25°C. *P. aciculare* and *D. dicarpum* indicated generally increasing nitrogen mineralisation for the whole material but after a slight peak at 30 days there was a general decrease in rate for ground material. This again suggests that the ground material was exposing substances that were readily utilised by the microbial population but once that substance was metabolised there was a decrease in activity.

The decrease in the rate of mineralisation could be due to immobilisation of the nitrogen, that is the nitrogen has either been incorporated into an organic form (such as in microbial biomass) or has been maintained in an organic form (Swift *et al*, 1979). If net immobilisation exceeds net mineralisation then the availability of the nitrogen to decomposer organisms will be limited (Swift *et al*, 1979; Richards, 1987). This will result in reduced biomass production by the decomposer organisms.

The green and brown portions of each species at both temperature treatments exhibited the same trends, but the percentages did vary with the brown (older) region having a greater percent of nitrogen mineralised. The increased rate of mineralisation from the older moss material suggests that there is a higher concentration of easily decomposable compounds present. This is probably due to the older regions containing senescent and dead material, which is more susceptible to degradation This material contains cells which are metabolically inactive and have experienced chemical alterations to the constituents of the cells which cause the material to be more vulnerable to microbial attack (Larcher, 1995).

Temperature did not appear to have a significant affect on the rate of nitrogen mineralisation for any of the litter types.

4.4.1 Nitrification

Nitrification is the mineralisation process whereby nitrifying bacteria (*Nitrosomonas* and *Nitrobacter*) convert ammonia (NH_3) into nitrite (NO_2^-) and then nitrate (NO_3^-). Nitrite is usually rapidly converted into nitrate and therefore is not usually detected. The detection of nitrate in my study suggests that nitrifying microorganisms were proliferating and in some cases were dominating the mineralisation activity, as the nitrate levels were higher than the ammonium levels.

There was an indication of nitrate presence in some samples, which in some cases exceeded the amount of ammonium ions. Tables A.4 and A.4.1 show the amount of

nitrate mineralised as a percent of the total nitrogen mineralised. These results indicate that in some cases nitrate constituted up to 100% of the nitrogen mineralised.

There was generally more nitrate (NO_3^-) at 25°C than at 10°C for both the ground and whole litter. This can be illustrated by comparing the mean values for the green and brown fractions over the 180 days (Tables 10 and 11). The ground litter (green and brown) ranged from 1.9% to -0.1% of the initial nitrogen content for 180 days at 10°C, whereas the ground litter at 25°C ranged from 3.0% to 0.1%. In the case of the whole litter no nitrate was detected from either litter type at 10°C. At 25°C, however, the means for the whole litter (green and brown) ranged from 0.2% to 2.0% over 180 days.

The generally higher nitrate levels at 25°C for all treatments suggests that nitrifying microorganisms are adapted to temperatures which are closer to 25°C than 10°C, and therefore were not as active at the lower temperature.

Differences in nitrate present between the green and brown fractions were also observed. The green litter (ground) exhibited higher nitrate levels initially (1.9% and 3.0% of the total sample nitrogen for both temperatures respectively at 30 days) but after 90 days larger values were obtained from the brown (ground) material. After 180 days there was little or no nitrate detected from either litter type. The means at 25°C for the green (whole) litter were higher for the first two sampling periods (0.6% and 1.1% respectively) but were slightly lower than the brown after 180 days (1.8%). The whole litter at 10°C exhibited little or no nitrate levels over the 180 days.

Over time it was also observed that less NO_3^- was mineralised from ground samples, whereas NO_3^- release from whole samples increased. The indication that the amount of nitrate released from ground litter was the opposite trend to the whole litter could be due to the influence of breaking down the plant material before inoculation. The ground litter had the highest nitrate levels initially and then the levels decreased, this could be a result of having the substrate compounds more readily available and as time progressed the less readily accessible compounds became more prevalent. In the

case of the whole litter the material had not been broken down prior to incubation and therefore the microorganisms may have had to degrade more of the moss structure before the appropriate substrates were made available to the nitrifying microorganisms.

The total nitrogen mineralisation rates exclude nitrogen that was mineralised and immediately assimilated into microbial biomass, rather than being released. Observation of the extent of microbial growth, however, gives an indication of the microbial biomass present in the microcosms and an increase in pH can be used to suggest the extent of decomposition that has occurred. The nitrogen content assimilated by the microbial population could be quantified by determining the nitrogen content of the microbial tissue. This was not calculated for the microcosms due to the lack of accuracy in determining the microbial biomass.

Other litter types such as flowers and bark do not exhibit similar nitrogen mineralisation levels over the first 200 days of decomposition. (Smaill, 2001) indicated that *Nothofagus fusca* floral litter (whole) at 25°C reached a maximum of 0.5% over 200 days incubation and a maximum of 3.8% at 10°C. All forms of *Nothofagus fusca* bark litter exhibited negative mineralisation at both temperatures over a 200 day period. This suggests that moss material is a more desirable substrate than bark but is of a lower quality than floral litter at 10°C.

4.5 Litterbags

Containment systems such as litterbags have been widely used in decomposition studies of many species and litter types (Heal *et al*, 1997). The technique is simple and convenient but it comes with limitations as well. The data obtained from litterbags is limited to net change within the container and ignore the fate of the material, which has been released. The use of single species (in many studies) not natural mixtures and microclimatic effects of the litterbags are also limitations of the technique. A further restriction on gaining data from containment studies is the exclusion of fauna and vegetation, which may impact on the rate of decay in a natural

system. The use of litterbags is not condemned as long as the limitations are recognised (Heal *et al*, 1997). Another problem encountered with litterbag studies is the contamination and/or physical loss of particles being recorded as weight loss.

The litterbags when removed from the soil, in which they were buried, were intact, moist and on closer inspection there was no obvious interference by soil animals. This observation suggests that the soil animals did not graze on the moss litter. There was also little or no microbial growth observed in the litterbags. Any microbial growth observed was not removed from the litter and so the mass loss values are probably slight underestimates. The litter had not changed in appearance and did not seem to have decayed.

The results are relatively inconclusive as in many cases there was negative weight loss, which could be due to the litter growing or to errors in the weighing of the bags. The litter was heated to kill the samples therefore theoretically the litter should not have grown but the increase in weight suggests that the litter may not have been heat killed. The ash content could indicate soil contamination, which may also have caused an increase in weight. The ash content determined before and after incubation did not differ substantially, which indicated that there was no soil contamination. Due to the lack of soil contamination, the fact that the litter should have been heat killed and the lack of considerable microbial growth the results obtained are very difficult to interpret.

The results do suggest that microbial attack was minimal over the time period and was not as extensive as in the microcosm experiment. Further work using litterbag techniques would be beneficial to giving a general overview of microbial breakdown in the environment.

Much of the past research has studied moss decomposition via litterbag experiments. In some cases the litter has been known to grow (Van Tooren, 1988) which is a difficulty encountered with moss material as it regenerates easily and it is difficult to distinguish whether it is dead material or not. Studies have indicated generally slow

rates of decomposition in grasslands, bogs, forests, Antarctic and Arctic tundra. Generally after a year little decomposition had occurred (approximately less than 20%) and the litter still appeared intact. Many papers have noted that it can take 2 years and upward before any significant decay begins (Clymo, 1965; Berg, 1984; Smith and Walton, 1986; Van Tooren *et al*, 1987; Van Tooren, 1988; Rochefort *et al*, 1990; Johnson and Damman, 1991; Hobbie, 1996). Initial decay is linked to leaching and the subsequent decreased rate is suggested to be due to low litter quality (such as high C:N, C:P ratios), inhibitory compounds such as antimicrobial substances or to recalcitrant molecules such as polyphenols.

4.6 Summary

The limitations of studying litter decomposition via both laboratory and litterbag techniques are acknowledged and the results presented in this research are by no means indicative of all the influences on microbial decomposition of bryophyte litter. The results give an indication of the rates at which decomposition may occur under the conditions stated in the study.

In vivo experiments allow the study of specific aspects of the decomposition cycle, such as particular temperatures, moisture regimes and litter types. This form of study indicates the influence that such factors have in a controlled environment. It would be difficult to study these individual factors in the field, as it would be impossible to control all the factors involved. In vitro experiments however do give the researcher an idea of the likely field situation and from that data one can predict decomposition rates and nitrogen release rates in the field. There are limitations in both techniques but, combined, the data from both situations can start to create a picture of what the real situation may be and also give an idea of how important various physical, chemical, and biological factors are in the environment.

The carbon and phosphorus content of the moss species indicated that the C:N and C:P ratios were generally high and the nitrogen distribution suggested that much of the acid soluble nitrogen originated from amino acids and proteins. The high

proteinaceous content does not necessarily indicate high litter quality, however as the proteinaceous material may be protected and therefore resistant to degradation. There did not appear to be any significant difference between the chemical content of the green and brown fractions.

From the experimental undertaken in this study it can be suggested that the rate of decomposition exhibited by selected New Zealand bryophyte species is slow and this slow rate is probably partly due to the high C:N and C:P ratios and the possible presence of "lignin-like" or polyphenolic compounds. The mineralisation results over 180 days indicate that there is not a high rate of microbial activity occurring. Although the rates were generally low the higher mineralisation rate for ground litter, which is less likely to occur in nature, suggests that nitrogen mineralisation in the field is probably considerably lower. The amount of nitrogen leached was low compared with other litter types such as branch bark (44%) and old stem outer bark (35%) (Smaill, 2001). This suggests that the rate of loss from leaching would not be high, therefore, additional nutrient loss from leachates would probably not be a large influence on the rate of nutrient flux. The higher soluble nitrogen values coupled with the slowed decomposition rates suggests that initial losses of nitrogen through the physical process of leaching could be large but subsequent removal will probably be slowed due to the recalcitrant nature of the litter.

Biomass data was not collected and was unavailable through literature searches. This data would give an indication of what proportion of the system consisted of bryophyte species and allow an estimation of the amount of nitrogen stored in the plant material. Litter fall data would also be of use, however, this data is difficult to obtain as bryophyte material does not obviously shed senesced material as trees and flowering plants do.

Due to logistical reasons the use of soil from the Cass and Maruia sites to create the inoculum was not utilized in the microcosm experiments. The use of the soil from the sites could have given an indication of whether the soil population varied and whether the variation caused effect on the rate of decomposition of the various moss species.

The influence of mosses in their natural environments is still poorly understood, but it can be suggested from past research and the results from the current study that they probably have a significant impact on nutrient cycling and energy flow in their respective ecosystems. Bryophytes are likely to be acting as slow release fertilizers in their environments as they are known to intercept nutrients from atmospheric deposition and the substratum which is then released slowly over time as the accumulated material decomposes (Oechel, 1986). Mosses probably not only govern nutrient loss from the system but also influence the community structure of the environment as they can influence seedling germination, create sites of nutrient accumulation and alter the physico-chemical environment such as pH, temperature and moisture. To make more conclusive statements about the role of bryophytes in nutrient dynamics further work on biomass data, litter fall and litter accumulation is required. A longer and fuller field study would also be beneficial

Although the results for the litterbag experiment were not conclusive, it does indicate the need for further study in the natural environment and highlights the difficulties of litterbag field studies. Bryophytes are resilient plants and tend to be able to survive adverse conditions such as very high or very low (e.g. Antarctica and Arctic) temperatures. As many moss species are able to desiccate they can withstand high temperatures. In fact they essentially become thermophilic in many cases, therefore it may take temperatures of 90°C and greater to kill the samples if they have already been air-dried (Zeiger, 1991). The resilience of the material causes difficulties when studying parameters such as weight loss as the material may grow. The usual technique, therefore, is to heat kill the litter. The samples in the bags had been heated to high temperatures (~ 90°C) to heat kill the material and perhaps chloroform could be used as an alternative treatment in future since it effects are not as harsh as those associated with heat.

4.7 Future work

Further study is required to continue the collection of data on moss decomposition and the role that mosses have in nutrient cycling within an environment. In particular

research into Southern Hemisphere mosses other than Antarctic species needs to be undertaken, as there is a distinct lack of published information.

Further study into decomposition via mass loss and mineralisation would add to the findings of this thesis, which in turn would add to the general information pool. Any further study should include a larger *in situ* experiment than has been achieved in the above experimental to indicate the *in situ* losses over a longer time period. Placement of litterbags at the same time would allow studies of the influence of climate on litter decay. Due to logistical reasons larger replication was not used in the above study however it is recommended in further research to increase replication which will in turn increase the statistical significance of the results.

Repetition of the above experiment to include a larger range of elements such as phosphorus, potassium and calcium would extend the range of findings and make them more compatible with other litters.

Prolonged nutrient release studies could be undertaken to obtain further information about nitrogen dynamics (leaching, accumulation and final release) as have been suggested by Berg and Staaf (1981).

Further investigation of the chemical composition of moss litter such as the polyphenol and "lignin-like" content would aid in defining the resource quality of bryophyte material.

The common chemicals present in the water-soluble extracts need to be determined. Knowledge of the chemical constituents of the extracts could allow an indication of why the extracts are easily broken down and whether there are any substances present that are inhibitory to microorganisms or invertebrates.

The collection of biomass data would contribute to the decomposition data and allow the rate of moss turnover to be determined.

The suggested areas of study mentioned in this section would extend the work from this study and give further evidence of the influence bryophyte decomposition has on the ecosystems within New Zealand.

Acknowledgments

I would like to thank my supervisor, Dr Laurie Greenfield, for the help and encouragement he has provided during the course of my research.

A thanks also goes out to Allan Fife for willing to be my co-supervisor and aiding in the identification of the moss species.

To Craig Galilee, Analeise Murahidy, Steve Kroening and Simeon Smaill (from my lab) I would like to say a big thank you for the support and encouragement and for keeping me sane when I needed it the most.

To all my friends (especially Jo and Andy) and my enduring flatmates (Kate, Mark and Marie) I would like to let you know that the love and support you gave was appreciated more than I can say.

To Mum, Dad, Alistair and Rosie I would like to say a huge thank you not only for the financial support but also for all the encouragement you gave especially when I doubted my self.

Finally I would like to thank my late Grandad for believing in my abilities, encouraging me to make the most of myself and to not give up.

References

Asakawa, Y. 1990. Biologically Active Substances from Bryophytes. In: R. N. Chopra, and Bhatla, S.C. (eds.), *Bryophyte Development: Physiology and Chemistry.* Boca Raton, Florida: CRC Press, Inc.: pp. 259-287.

Beever, J., Allison, K.W., and Child, J. 1992. *The mosses of New Zealand.* Dunedin, University of Otago Press. 4-5, 32, 47, 105, 115, 150 pp.

Berg, B. 1984. Decomposition of moss litter in a mature Scots pine forest. *Pedobiologia* 26: 301-308.

Berg, B., and Staaf, H. 1981. Leaching, accumulation and release of nitrogen in decomposing forest litter. In: F. E. Clark, and Rosswall, T. (eds.), *Terrestrial nitrogen cycles.* 33: pp. 163-178.

Bremner, J.M. 1965a. Inorganic forms of nitrogen. In: C. Black (eds.), *Methods of soil Analysis part 2. Chemical and Microbiological Properties.* Madison, USA: American Society of Agronomy, Inc. 2: pp. 1179-1237.

Bremner, J.M. 1965b. Organic forms of nitrogen. In: C. Black (eds.), *Methods of Soil Analysis part 2. Chemical and Microbiological Properties.* Madison, USA: American Society of Agronomy Inc. 2: pp. 1238-1255.

Brown, D., and Bates, S. 1990. Bryophytes and nutrient cycling. *Botanical Journal of the Linnean Society* 104: 129-147.

Burgess, J. 1985. *Introduction to plant cell development.* Cambridge, Cambridge University Press. 176-177

Burrows, C.J. 1977. Grassland vegetation. In: C. J. Burrows (eds.), *Cass.* Christchurch: Department of Botany, University of Canterbury: pp. 185.

Chapin III, F., Oechel, W.C., Van Cleve, K., and Lawrence, W. 1987. The role of mosses in the phosphorus cycling of an Alaskan black spruce forest. *Oecologia* 74: 310-315.

Clymo, R.S. 1965. Experiments of breakdown of *Sphagnum* in two bogs. *Journal of Ecology*: 747-758.

Coulson, J., and Butterfield, J. 1978. An investigation of the biotic factors determining rates of plant decomposition on blanket bog. *Journal of Ecology* 66: 631-650.

Davidson, A.J., Harborne, J.B., and Longton, R.E. 1990. The acceptability of mosses as food for generalist herbivores, slugs in the Arionidae. *Botanical Journal of the Linnean Society* 104: 99-113.

Davis, R.C. 1986. Environmental factors influencing decomposition rates in two Antarctic moss communities. *Polar biology* 5: 95-103.

DeAngelis, D.L. 1992. *Dynamics of nutrient cycling and food webs.*, London, Chapman and Hall. 8-10 pp.

Doyle, W.T. 1970. *Nonseed plants: Form and function.* Belmont, Wadsworth publishing company, Inc. 170-171, 176 pp.

Erickson, M., and Miksche, G.E. 1974. On the occurance if lignin or polyphenols in some mosses and liverworts. *Phytochemistry* 13: 2295-2299.

Farrish, K.W., and Grigal, D.F. 1985. Mass loss in a forested bog: Relation to hummock and hollow microrelief. *Canadian Journal of Soil Science* 65: 375-378.

Frankland, J. 1974. Decomposition of lower plants. In: G. J. F. Dickinson C.H. and Pugh (eds.), *Biology of plant litter decomposition.* London: Academic press: pp. 1-36.

Gerson, U. 1982. Bryophytes and invertebrates. In: A. J. E. Smith (eds.), *Bryophyte ecology.* London: Chapman and Hall Ltd: pp. 291-332.

Gosz, J.R., Likens, G.E. and Bormann, F.H. 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook forest, New Hampshire. *Ecological monographs* 43: 173-191.

Greenfield, L. 1992. Nitrogen analysis of New Zealand and Antarctic lichens. *Lichenologist* 24 (4): 377-381.

Greenfield, L.G. 1999. Weight loss and release of mineral nitrogen from decomposing pollen. *Soil biology and biochemistry* 31: 353-361.

Hattenschuiler, S., and Vitousek, P. 2000. The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends in ecology and evolution* 15 (6): 238-243.

Heal, O.W., Anderson, J.M., and Swift, M.J. 1997. Plant litter quality and decomposition: An histroical overview. In: G. Cadish, and Giller, K.E. (eds.), *Driven by nature: Plant litter quality and decomposition.* Wallingford, UK: CAB International: pp. 3-31.

Hesse, P. 1971. Carbon and organic matter. (eds.), *A textbook of soil chemical analysis.* London: John Murray Publisher: pp. 204-254.

Hobbie, S. 1996. Temperature and plant species control over litter decomposition in Alaskan tundra. *Ecological monographs* 66 (4): 503-522.

Hodkinson, I.D. 1975. Dry weight loss and chemical changes in vascular plant litter of terrestrial origin, occurring in a beaver pond ecosystem. *Journal of Ecology* 63: 131-142.

Hogg, E., Malmer, N., and Wallen, B. 1994. Microsite and regional variation in the potential decay of Sphagnum in south Swedish raised bogs. *Ecography* 17: 50-59.

Howard, P.J.A., and Howard, D.M. 1974. Microbial decomposition of tree and shrub leaf litter. I. Weight loss and chemical composition of decomposing litter. *Oikos* 25: 341-352.

Johnson, L., and Damman, A. 1991. Species controlled Sphagnum decay on a south Swedish raised bog. *Oikos* 61: 234-242.

Keenan, R.J., Prescott, J.P., Pastor, J., and Dewey, B. 1996. Litter decomposition in western red ceder and western hemlock forests on northern Vancouver Island, Bristish Columbia. *Canadian Journal of Botany* 74: 1626-1634.

Keizer, P.J., Van Tooren, B.F. and During, H.J. 1985. Effects of bryophytes on seedling emergence and establshment of short-lived forbs in chalk grassland. *Journal of Ecology* 73: 493-504.

Killham, K. 1994. *Soil ecology.* Cambridge, Cambridge University Press. 102-103, 116-117 pp.

Kitson, R.E., and Mellon, M.G. 1944. Colorimetric determination of phosphorus as molybdivanadophosphoric acid. *Industrial and engineering chemistry* 16 (6): 379-383.

Krassig, H., Schurz, J., Steadman, R., Schliefer, K. and Albrecht, W. 1996. Cellulose. In: W. Gerhartz, Yamamoto, Y., Cambell, T., Pfefferkorn, R., and Rounsaville, J. (eds.), *Ullman's encyclopedia of industrial chemistry.* New York, USA: VCH Publishers. A5: pp. 375-418.

Larcher, W. 1995. *Physiological plant ecology.*, Berlin, Germany, Springer-Verlag, 298-300 pp.

Latter, P.M., and Cragg, J.B. 1967. The decomposition of *Juncus squarrosus* leaves and microbiological changes in the profile of *Juncus* moor. *Journal of Ecology* 53: 465-483.

Longton, R.E. 1984. The role of bryophytes in terrestrial ecosystems. *Journal of the Hattori botannical laboratory* 55: 147-163.

Longton, R.E. 1992. The role of bryophytes and lichens in terrestrial ecosystems. In: J. W. Bates, and Farmer, A.M. (eds.), *Bryophytes and Lichens in a changing environment.* Oxford: Oxford Science Publications, Oxford University Press.: 32-61 pp.

MacLean, D.A., and Wein, R.W. 1978. Weight loss and nutrient changes in decomposing litter and forest floor material in New Brunswick forest stands. *Canadian Journal of Botany* 56: 2730-2749.

Meentemeyer, V. 1978. Macroclimate and lignin control of litter decomposition rates. *Ecology* 59: 465-472.

Nicholson, G. 1984. *Methods of soil, plant and water analysis.* Soils and Site Amendment Section, Rotorua, New Zealand. 17-19 pp. (Forest Research Institute Bulletin no. 70)

NIWA 2000. *NZ Climate digest.*

Nykvist, N. 1959. Leaching and decomposition of litter. *Oikos* 10 (2): 190-211.

Olson, J. 1963. Energy storage and the balance of producers and decompsoers in ecological systems. *Ecology* 44: 322-331.

Pelczar Jr, M.J., Chan, E.C.S. and Kreig, N.R. 1993. *Microbiology: Concepts and applications.* New York, McGraw-Hill Inc. 785-786 pp.

Prins, H.H. 1982. Why are mosses eaten in cold environments only? *Oikos* 38 (3): 374-380.

Proctor, M.C.F. 1982. Physiological ecology: Water relations, light and temperature responses, carbon balance. In: A. J. E. Smith (eds.), *Bryophyte ecology.* London: Chapman and Hall Ltd: pp. 333-383.

Rice, E.L. 1984. *Allelopathy.* Orlando, Florida, Academic Press, Inc. 1,6 pp.

Richards, B.N. 1987. *The microbiology of terrestrial ecosystems,* Longman Scientific and Technical, Longman Group UK Limited. 2, pp.

Rochefort, L., Vitt, D., and Bayley, S. 1990. Growth, production and decomposition dynamics of Sphagnum under natural and experimental acidified conditions. *Ecology* 71 (5): 1986-2000.

Round, F.E. 1969. *Introduction to lower plants.* London, Butterworth and Co. (Publishers) Ltd. 112 pp.

Russell, S. 1990. Bryophyte production and decomposition in tundra ecosystem. *Botanical journal of the Linnean Society* 104: 3-22.

Satchell, J.E. 1974. Litter-Interface of animate/inanimate matter. In: Dickinson C.H. and Pugh G. J. F. (eds.), *Biology of plant litter decomposition.* London: Academic press: pp. XiV-Xl.

Shanks, A., Glenny. D., Gibson, R., Rosser, K., Roozen, D., Phillipson, S., Steven, J., and Arand, J. 1990. Coleridge, Craigieburn and Cass ecological districts-Survey report for the Protected Natural Areas Programme. Wellington, Department of Conservation.

Smaill, S.J. 2001. The decomposition of *Nothofagus fusca* floral and bark litter. Thesis. Plant and Microbial Sciences, University of Canterbury. pp.1-120.

Smith, M., and Walton, D. 1986. Decomposition of grass and moss litter in subantarctic tundra. *Pedobiologia* 29: 193-200.

Staaf, H.a.B., B. 1977. Mobilisation of plant nutrients in a Scots pine forest mor in Central Sweden. *Silva Fennica* 11: 210-217.

Swift, M.J., Heal, O.W., and Anderson J.M. 1979. *Decompositon in terrestrial ecosystems*. Oxford, Blackwell scientific publications. 34-35, 37, 49-53, 56-57, 66-69, 118-123, 220-247 pp.

Taylor, B.R., Parkinson, D., and Parsons, W.F.J. 1989. Nitrogen and lignin content as predictors of litter decay rates: A microcosm test. *Ecology* 70 (1): 97-104.

Van Tooren, B.F. 1988. Decomposition of bryophyte material in two Dutch chalk grasslands. *Journal of Bryology* 15: 343-352.

Van Tooren, B.F., den Hertog, J., and Verhaar, J. 1987. The role of bryophytes in a chalk grassland ecosystem. *Symposia Biologica Hungarica* 35: 665-675.

Wardle, D.A., and Laville, P. 1997. Linkages between soil biota, plant litter quality and decomposition. In: Cadish, G. and Giller, K.E. (eds.), *Driven by nature: Plant litter quality and decomposition.* Wallingforn, UK: CAB International: pp. 107-124.

Wardle, D.A., Nilsson, M., Gallet, C., and Zackrisson, O. 1998. An ecosystem-level perspective of allelopathy. *Biological Review* 73: 305-319

Watt, A.S. 1947. Pattern and process in the plant community. *Journal of Ecology* 35: 1-22.

Weetman, G.F 1968. 3rd International Peat Congress, Quebec: pp.366-370

Williams, S.T., and Gray, T.R.G. 1974. Decomposition of litter on the soil surface. In: Dickinson C.H. and Pugh, G. J. F (eds.), *Biology of plant litter decomposition.* London: Academic Press: pp. 611-632.

Zeiger, T. 1991. Stress physiology. In: Taiz, L. and Zeiger, E. (eds.), *Plant physiology.* California: The Benjamin/ Cummings Publishing Company, Inc.: pp. 360.

Appendices

A.1 Phosphorus Analysis Calibration Curve

The absorption readings from standard phosphorus solutions with known concentrations are given in Table A.1. A regression equation relating solution absorbance to phosphorus concentration was formulated. See equation 6.

Equation 1

$$\text{PPM Phosphorus} = (0.0176 \times \text{Adjusted reading}) - 0.0329$$

R^2 for this regression was 0.972, indicating the equation was highly linear.

Table A.1 Phosphorus calibration curve data

PPM Phosphorus	Reading	Adjusted Reading
0	9.825	0
4	9.84	0.015
8	9.93	0.105
10	9.95	0.125
12	9.99	0.165
16	10.085	0.26
20	10.16	0.335
24	10.259	0.434
32	10.413	0.588

A.2 Average pH for the soil solution in the mineralisation microcosms

The average pH of the soil solution at both temperature treatments over the 180 study period for both ground and whole samples are shown in Tables A.2 and A.2.1.

Table A.2 Average pH at 10°C and 25°C over the 180 day study period, ground samples.

Ground	10°C			25°C		
Sample	**30**	**90**	**180**	**30**	**90**	**180**
Green						
P. aciculare	6.7	6.7	7.4	6.8	5.5	7.3
H. comatum	8.4	7.7	7.8	7.5	5.2	5.1
D. dicarpum	6.9	6.9	7.0	7.4	6.9	7.1
H. cupressiforme	5.8	ND	7.5	6.9	ND	6.8
B. pendula	6.7	6.4	8.0	7.4	6.7	7.2
R. pruinosum	5.7	6.9	7.3	6.0	5.7	7.0
Brown						
P. aciculare	6.3	6.0	7.2	6.2	4.9	7.2
H. comatum	6.9	6.7	6.4	7.1	5.9	4.7
D. dicarpum	6.6	6.2	7.4	6.6	6.2	6.8
H. cupressiforme	6.0	ND	8.3	6.2	ND	6.9
B. pendula	6.5	4.3	8.2	6.7	5.0	6.2
R. pruinosum	5.3	6.6	7.9	5.5	5.6	7.6

Note: ND these species were not analysed due to logistical reasons

Table A.2.1 Average pH at 10°C and 25°C over the 180 day study period, whole samples.

Whole	10°C			25°C		
Sample	**30**	**90**	**180**	**30**	**90**	**180**
Green						
P. aciculare	5.7	6.7	6.3	4.7	5.4	5.0
H. comatum	ND	ND	7.4	ND	ND	5.0
D. dicarpum	5.7	6.3	6.9	6.1	5.5	6.1
H. cupressiforme	ND	ND	7.3	ND	ND	4.8
B. pendula	6.2	6.7	7.1	5.5	5.7	6.6
R. pruinosum	7.2	6.1	6.4	6.8	5.6	4.5
Brown						
P. aciculare	5.0	6.3	5.8	6.0	5.4	4.6
H. comatum	ND	ND	6.6	ND	ND	4.7
D. dicarpum	5.5	6.0	5.8	5.9	5.4	5.4
H. cupressiforme	ND	ND	7.0	ND	ND	4.7
B. pendula	6.2	6.9	5.0	5.2	5.2	5.8
R. pruinosum	7.1	6.5	5.8	7.4	5.6	5.0

Note: ND these species were not analysed due to logistical reasons

A.3 Microbial growth in the mineralisation microcosms

The average microbial growth over each triplicate in the ground litter microcosms is shown in Table A.3. There is no result for ground *H. cupressiforme* at 90 days as there was a lack of litter material. The unground average microbial growth is shown in Table A.3.1. Litter from two species from each site was used in the whole experiment due to a lack of litter for *H. comatum* and *H. cupressiforme.*

Table A.3 Average microbial growth at 10°C and 25°C over the 180 study period, ground samples.

Ground	10°C			25°C		
Sample	**30**	**90**	**180**	**30**	**90**	**180**
Green						
P. aciculare	+++++	+++++	++	++++	++	+
H. comatum	+++++	++++	+	+++++	+++	+++
D. dicarpum	++++	+++	++++	++	0	++
H. cupressiforme	+	ND	0	0	ND	0
B. pendula	++++	+++	+	+++	+	0
R. pruinosum	+	+	0	+	+	0
Brown						
P. aciculare	+++++	+++++	+	+++++	++	+
H. comatum	+++++	++++	++++	+	++++	++
D. dicarpum	+++++	+++	+	+++	0	0
H. cupressiforme	+++++	ND	0	0	ND	0
B. pendula	++++	+++	+	+++++	+	0
R. pruinosum	++	+++	0	+	0	0

Note: ND refers to species that were not analysed due to logistical reasons

Table A.3.1 Average microbial growth at 10°C and 25°C over the 180 study period, whole samples.

Whole	10°C			25°C		
Sample	30	90	180	30	90	180
Green						
P. aciculare	+++	+++	++++	++++	+++	++
D. dicarpum	+++	+++	++	++	++	++
B. pendula	++++	++++	++	+++	++	++
R. pruinosum	++++	++	++	++	++	+
Brown						
P. aciculare	++	+++	+++	+++	++	+
D. dicarpum	+++	++++	++	+++	++	++
B. pendula	+++	+++	++	++	++	+
R. pruinosum	+++	++	+	++	++	+

A.4 Nitrate levels detected in the mineralisation microcosms as a % of total nitrogen mineralised

Nitrate production as a percentage of the total nitrogen mineralised for all treatments are shown in Tables A.4 and A.4.1.

Table A.4 Nitrate production (as % of total nitrogen mineralised) for ground litter at 10°C and 25°C.

Ground	10°C			25°C		
	30 days	**90 days**	**180 days**	**30 days**	**90 days**	**180 days**
Green						
P. aciculare	14	29	0	65	100	0
H. comatum	38	8	0	49	100	100
D. dicarpum	45	0	0	74	2	0
H. cupressiforme	ND	ND	0	33	ND	0
B. pendula	72	0	0	4	60	0
R. pruinosum	55	0	0	0	15	4
Mean	**45**	**7**	**0**	**38**	**55**	**21**
Brown						
P. aciculare	69	24	0	73	100	0
H. comatum	20	22	0	27	100	97
D. dicarpum	22	0	0	0	35	0
H. cupressiforme	ND	ND	0	ND	ND	0
B. pendula	40	6	0	44	87	1
R. pruinosum	#	0	0	100	0	0
Mean	**38**	**10**	**0**	**49**	**64**	**20**

Note: ND refers to species that could not be analysed due to logistical reasons.

refers to values that could not be calculated.

Table A.4.1 Nitrate production (as % of total nitrogen mineralised) for whole litter at 10°C and 25°C.

Whole	10°C			25°C		
	30 days	**90 days**	**180 days**	**30 days**	**90 days**	**180 days**
Green						
P. aciculare	0	0	0	31	47	82
H. comatum	ND	ND	0	ND	ND	95
D. dicarpum	0	0	0	100	57	93
H. cupressiforme	ND	ND	2	ND	ND	90
B. pendula	0	0	0	70	76	100
R. pruinosum	0	0	0	3	40	100
Mean	**0**	**0**	**0.3**	**51**	**55**	**93**
Brown						
P. aciculare	0	0	0	22	33	89
H. comatum	ND	ND	0	ND	ND	79
D. dicarpum	0	0	0	100	35	100
H. cupressiforme	ND	ND	0	ND	ND	94
B. pendula	0	0	0	21	46	100
R. pruinosum	0	0	0	0	0	54
Mean	**0**	**0**	**0**	**36**	**29**	**86**

Note: ND refers to species that were not tested for logistical reasons.

A.5 Nitrogen mineralisation for *H. comatum* and *H. cupressiforme*

Total N mineralised (as % of original nitrogen content) after 180 days for whole *H. comatum* and *H. cupressiforme* litter. Only the 180 day incubation was set up due to logistical reasons.

Table A.5 Total N mineralised (as % of original nitrogen content) for whole litter after 180 days

Whole litter	**Total N mineralised (%) after 180 days**
Green 10°C	
H. comatum	1.1 ±0.07
H. cupressiforme	2.5 ±0.06
Brown 10°C	
H. comatum	0.7 ±0.09
H. cupressiforme	1.7 ±0.26
Green 25°C	
H. comatum	1.1 ±0.10
H. cupressiforme	3.9 ±0.29
Brown 25°C	
H. comatum	1.0 ±0.07
H. cupressiforme	3.4 ±0.33

A.6 Weight loss (%) for *H. comatum* and *H. cupressiforme* after 180 days

Weight loss (%) after 180 days for *H. comatum* and *H. cupressiforme* litter. The percentage of weight lost from *H. comatum* litter increased with increasing temperature from 10°C to 25°C. *H. cupressiforme* decreased as the temperature increased.

Due to logistical reasons these species were only analysed after 180 days.

Table A.6 Weight loss (%) after 180 days for *H. comatum* and *H. cupressiforme* litter

Whole litter	Weight loss (%) after 180 days
Green 10°C	
H. comatum	18±1.06
H. cupressiforme	17±0.42
Brown 10°C	
H. comatum	19±0.84
H. cupressiforme	17±2.04
Green 25°C	
H. comatum	25±2.19
H. cupressiforme	12±0.43
Brown 25°C	
H. comatum	21±0.84
H. cupressiforme	14±0.16